THE QUANTITATIVE AND QUALITATIVE ANALYSIS OF MICROORGANISMS EMITTED FROM THE ARMS AND HANDS OF DAIRY PLANT PERSONNEL

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

FE CORAZON A. SUNGA

1968

ABSTRACT

THE QUANTITATIVE AND QUALITATIVE ANALYSIS OF MICROORGANISMS EMITTED FROM THE ARMS AND HANDS OF DAIRY PLANT PERSONNEL

by Fe Corazon A. Sunga

A special two-compartment chamber was constructed to determine the extent the arms and hands of workers contribute to the air-borne microorganism population in a dairy plant. The Andersen (six-stage) sampler was used for enumerating the number of microorganisms shed. A Casella slit sampler was used for enumerating selected microorganisms such as probable staphylococci, hemolytic organisms and coliforms.

The number of microorganisms shed from the treated arms and hands (washed and sanitized) and untreated arms and hands (washed only) of four workers ranged from 0 to 93 and 0 to 95 per minute, respectively. Of the total viable particles shed, 55.08% were in the size range of 3.3 to 5.5 microns. Two hundred and fifty-six organisms were identified, of which 55.4% were cocci, 41.4% were rods and 3.2% were yeasts. The principal species identified were Sarcina flava, Peptococcus prevotii, Sarcina

aurantiaca, Sarcina hansenii, Staphylococcus epidermidis, Alcaligenes marshallii, Alcaligenes bookeri and Pseudomonas synxantha. The yeasts were probably saccharomyces and candida. Most of the microorganisms identified were native inhabitants of the skin, dairy products and utensils, water, soil, dust and intestinal canal.

All four subjects yielded small numbers of probable staphylococci. Two subjects shed hemolytic-type organisms. No coliforms were isolated from the microorganisms shed.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1968

ACKNOWLEDGEMENTS

The author wishes to extend her heartfelt gratitude to Dr. T. I. Hedrick (Department of Food Science) and Dr. D. R. Heldman (Departments of Agricultural Engineering and Food Science) for their invaluable guidance and advice. A special word of thanks to Dr. F. R. Peabody (Department of Microbiology and Public Health), member of the guidance committee. The author is also indebted to Dr. Cecilia A. Florencio for her constant help and encouragement.

This investigation was supported in part by Public Health Research Grant No. UI00284 from the National Center for Urban and Industrial Health.

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INTRODUCTION

The advent of aseptic packaging of various food products, i.e., sterilized milk, has focused attention to environmental microbiology. According to Greene (1), environmental microbiology deals with problems such as the survival of microorganisms in the environment, the source of microorganisms that are encountered in the environment, their generation from living and inanimate reservoirs, their dissemination, transportation and significance.

The air has long been recognized as one of the sources of contamination in dairy products. The air-borne organisms that get into a product can grow and alter its shelf life. Microorganisms that settle onto equipment or surfaces where there are moisture and nutrients will multiply and possibly become sources of air-borne contamination.

The only way to produce a product of excellent quality is to process it efficiently from high quality raw materials under sanitary conditions. Sanitation in the food industry should embrace two aspects: (a) the prevention of public health hazards and (b) the prevention of contamination with extraneous materials (2). Air-borne contamination and infection could certainly be involved in either of the above categories. Firstly, air-borne contam-

ination could mean the addition of a viable microorganism to the food. Infection could occur with the proper conditions for growth of the microorganisms. An increase in the number of microorganisms present in the food may hasten deterioration. Secondly, air-borne microorganisms may be considered as extraneous materials relative to the food product itself.

People are a source of contamination. The role of humans as contributors to air-borne contamination has been studied in hospitals, especially in operating rooms. In spacecraft sterilization, ultra-clean rooms have been used. This is to achieve aseptic handling of all components, through to the final assembly of the spacecraft. Human beings have been found to be the major source of contamination in industrial clean rooms. Recent studies have shown that shedding of organisms by different individuals may vary from thousands to millions of bacterial particles per minute. Up to the present, very few studies have been published on the role of human beings as sources of air-borne contamination in food processing areas.

The objective of this study was to determine the extent the arms and hands of workers in a dairy plant contribute to the air-borne microorganism population, by measuring the rate of shedding and identifying the major types of microorganisms isolated.

REVIEW OF LITERATURE

I. A Brief History of the Microbiology of Air

Air, a mixture of approximately 78 per cent nitrogen, 21 per cent oxygen and 0.035 per cent carbon dioxide with traces of inert gases, i.e., argon, helium, neon, krypton, and xenon varies in microbiological quality with time and location. The theory of air-borne infection had its beginning in 1546 when Girolamo Fracastoro published his germ theory of disease (3, 4). Fracastoro explained that "contagion is an infection that passes from one thing to another". He also enumerated three types of transmission: (a) by direct contact, (b) by fomites or indirect contact, (c) by transmission from a distance.

In 1799, Spallanzani performed a series of experiments in which he sealed vessels with tops of various degrees of porosity and showed that the number of microorganisms present was a function of the degree of porosity. Thus, he demonstrated the possibility that microorganisms were present in air (5).

Pasteur was inspired by Spallanzani's finding, and in 1861, he devised his own experiment (6). He observed that some flasks which contained nutrient medium remained

sterile when exposed to air while others did not. Another experiment which involved exposure of flasks at different places showed that the quantity of microorganisms varied from place to place. Later, Pasteur developed a controlled method of collecting particles from air. He extruded a tube through a window into the open. A plug of gun-cotton was placed on the back of the tube to catch the particles. A filter pump drew the air in and the volume of air was measured by displacement of water. The gun-cotton was dissolved in alcohol-ether solution and the residue was examined microscopically.

With Pasteur's discovery, interest in air microbiology grew. Pierre Miquel, a distinguished bacteriologist, did a survey of the microbiological content of the atmosphere in 1879 (6). He was the first to collect a known volume of air into sterile beef extract broth, and estimate the number of bacterial particles contained in the air sample. In 1885, in Paris, Miquel used paper discs soaked with nutrient agar to collect air-borne bacteria. He noted the daily occurrence of high and low bacterial counts. The period of high counts was closely related to activities in the city such as sweeping the street and the passage of horse-drawn traffic.

At the same time, similar work was in progress in Germany (6). Hesse devised a tube for sampling air which contained a layer of Koch's nutrient gelatin. After a known volume of air was blown slowly through the tube, microorgan-

isms grew on the medium. Observing that bacteria grew nearer the end of the tube while molds penetrated further into the tube, Hesse concluded that bacteria were heavier than molds.

The number of organisms in the air during different seasons of the year was also studied. Several workers (7, 8) found that smaller numbers of microorganisms were present in air during the winter and large numbers during the summer.

Wells, in 1934 introduced the concept of the droplet-nuclei mode of infection. He supported his theory by atomizing various pathogenic bacteria into a chamber and recovering viable organisms after hours and days (9). Currently the research involves principally air-borne infections, environmental sampling of air, dissemination of microorganisms by human beings, and various new sampling techniques and devices.

II. Environmental Air Sampling

Bacterial Air Sampling Techniques

A comprehensive review of the methods and description of the equipment for sampling microbiological aerosols was published by the Public Health Service (10). Therefore, only a limited treatment of the subject matter will be made. Among the methods commonly used at present are: (a) impingement in liquids, (b) impaction on solid surfaces,

(c) filtration, (d) sedimentation, (e) centrifugation, (f) electrostatic precipitation, and (g) thermal precipitation.

Liquid Impingement

Liquid impingers involve the trapping of bacterial particles in a liquid medium by high or low velocity impingement. In high velocity impingers, the air sample is drawn through a small jet and is directed against the surface of the liquid. In low velocity impingers, the air sample enters through a larger jet, a fritted glass disc, or a perforated tube. The particles are washed out of the air and the liquid is diluted as required and assayed. The bacterial clumps are usually broken up, especially with the high velocity impingers due to agitation. Therefore, the counts obtained indicate the total number of viable bacterial cells in the air sample.

May and Harper (11, 12) have enumerated the useful features of liquid impingers as follows:

(a) it is compact and inexpensive;
(b) the sample fluid can be plated out simultaneously on different media;
(c) an extreme range of air-borne concentration can be coped with by the serial dilution technique;
(d) virus aerosols may be estimated;
(e) it has a high efficiency for particle retention; traps all particles to about 0.5 micron;
(f) it gives a measure of the number of individual viable organisms in an aerosol;
(g) it acts as its own constant-flow metering device;
(h) it is unaffected by repeated autoclaving.

Inspite of these special features, liquid impingers also have their limitations (12). Among these are:

(a) it is not good with highly dilute aerosols;
(b) the liquid evaporates quickly, being under low pressure and tends to freeze in cool, dry air;
(c) the violent impingement or agitation can kill sensitive cells.

Liquid impingers using high velocity include: the Shipe or tangential jet (13); the cell-glass impinger (14, 15), critical orifice impinger (16). Examples of those impingers using low velocity are: the Greenburg-Smith impinger (17), M.S.A. midget impinger (18), the Folin bubbler (19, 20), and the Venturi scrubber (21, 22).

Solid Impaction

Impactors are samplers which collect the air-borne particles directly on solid agar surface. After incubation, the bacterial count obtained represents the number of viable air-borne bacterial particles. The particle size distribution can also be determined with this device. Examples of solid medium impactors are: the Andersen sampler (23, 24, 25), the sieve device (26), and the slit device (27, 28, 29, 30, 31, 32).

Solid impactor samplers have certain advantages (23, 26, 27, 28, 32, 33, 34):

(a) they are quick and simple to use;
(b) they determine the quantitative estimate of air-borne bacteria, if the volume of air sampled is measured accurately;

(c) they determine the particle size distribution of air-borne microorganisms;
(d) they collect the finest bacteria-carrying particles as efficiently as coarser ones.

The disadvantages of solid impactor samplers are:

(a) long sampling periods may cause dessication and death of certain organisms on agar surfaces;
(b) overcrowding of viable particles on the agar surface occurs if the air-borne concentration of particles is high.

Filtration

In the filtration technique, the particles are either collected on the filter or attracted to the filter by electrostatic forces (35, 36, 37, 38). To assay, either the filter or part of it is agitated in liquid medium and aliquots of this are plated out. Another simple method is soaking the filter paper in the desired medium and then incubating it (35). Various filter materials have been used such as cellulose-asbestos paper, glass wool, cotton, alginate, wool, gelatin foam and membrane filters.

Some advantages of filtration are:

(a) the particles present can be counted as in a normal slide preparation for the microscope;
(b) the membranes may be redissolved and cast in thin films with the retained particles and subjected to analysis through an electron microscope (38, 39).

The disadvantages of this method are: (a) higher bacterial counts are obtained than the actual viable number of particles due to agitation during the assay, and (b) death of some organisms due to dessication if the sampling period is too long.

Sedimentation

Sedimentation is a simple method of sampling microbiological aerosols. The suspended particles in the air are allowed to settle on plain surfaces or on nutrient agar surfaces. This method determines the number of viable particles or organisms, and the size of all particles that settle in a given time. The advantages of sedimentation samplers are: (a) inexpensiveness, (b) simplicity in use, (c) ability to collect the particles in their original state. The disadvantages are: (a) inability to give quantitative counts unless the aerosol sample is allowed to settle in a closed container, (b) a long waiting period required for all particles to settle, (c) some loss in viability occurring during the settling process (10, 40).

Centrifugation

Centrifugation works on the principle that when the aerosol moves in a circular path at high velocity, the suspended particles are impacted on the collecting surface by a force proportional to the particle velocity and mass (10). The most common samplers of this type are the Wells air centrifuge (41,42) and the cyclone (43). Very little use has been made of these types of samplers in published reports.

Electrostatic and Thermal Precipitation

Electrostatic precipitation and thermal precipitation samplers seldom have been used in sampling microbiological aerosols. This is due to the fact that they are complex

and require careful handling (10). Electrostatic precipitation works on the principle that electrically charged particles are attracted towards the oppositely-charged agar surface for collection (44). The thermal precipitation method of sampling involves the precipitation of small air-borne particles on a cold surface from the influence of a warmer surface (45, 46).

Air Disinfection and Sterilization

Measures advocated for the control of air-borne infection are classified into two categories (47):

(a) those designed to prevent dispersal of infectious material into the air;
(b) those employed for reducing the infectivity of already contaminated atmospheres, by killing or removing the disease-producing agents.

Methods of dust suppression like oiling of floors, blankets and bedding have been resorted to for preventing the dispersal of infectious material into the air (48, 49). The destruction or removal of microorganisms in the air may be effected by methods such as ultraviolet irradiation, filtration, chemical treatment, heat and electrostatic precipitation (50).

The chemical must be able to make contact with the air-borne microorganisms in order to be an effective aerial germicide or disinfectant. They are, therefore, either in the gaseous or vapor state. Some of the substances that have been tested and used are hypochlorous acid gas (51, 52), hypochlorite mists (52), lactic acid (53), iodine (54),

formaldehyde (55), propylene or ethylene glycol vapors (56, 57, 58, 59), ethylene oxide (55, 60), peracetic acid (61), and beta-propiolactone (61).

Propylene Glycol

Propylene glycol was used in the experiment and will be dealt with in more detail. The Merck Index of Chemicals and Drugs (62) lists "as mist to disinfect air" as one of the uses of this hygroscopic, viscous liquid. Glycol is relatively non-toxic and the vapor may be breathed without danger (63). It is the chemical which is most adaptable for general use because of its high bactericidal and virucidal potencies, its reasonable cost, its freedom from odors, toxicity and corrosiveness to metal surfaces (50). The mechanism of action of glycol vapors has been studied extensively. The lethal action of glycol vapor is due mainly to the liberation of gas by rapid evaporation of the aerosol droplets (57, 64). Robertson (57) concluded that the germicidal activity of glycol depended upon its relative saturation in the air rather than total concentration. Glycol vapors are most effective between 45 to 70% relative humidity with an optimum at 58% and a temperature below 80°F (56, 65).

Different methods of applying propylene glycol have been used. Among these are boiling at constant temperatures and atomization (66). Three recommended methods by Robertson, et al. (57) were: (a) place the glycol in petri dishes on

the floor of the room or chamber and allow it to evaporate overnight, (b) pour glycol, heated to 70° to 80°C, into petri dishes and place in the chamber. Then seal the chamber and allow to cool to room temperature with a fan circulating the air inside, (c) fill the chamber with air bubbled through propylene glycol which was heated to 60°C in a water bath. An instrument for maintaining the concentration of glycol vapors at a bactericidal but sub-fog level in inhabited rooms was devised by Puck, et al. (67).

Glycol vapors are more effective against small particles or droplet nuclei in the atmosphere than against dust particles (68). When dust particles were present, glycol vapors were more effective if used with dust suppressive measures (69). The advantage of using glycols is that the vapor permeates throughout the room and is not limited to the upper air like ultraviolet irradiation (70).

Filtration

Filtration involves the mechanical sieving of bacteria and other particles through fibrous granular material. Sykes (48) mentioned that: (a) the size of the particles to be filtered, (b) the diameter of the fibers or size of the granules constituting the filter material, and (c) the packing density of the filter, affect the efficiency of the filtration process.

Various materials such as glass wool, non-absorbent cotton, fiberglass and membrane filters have been used

(71, 72, 73). Sterilization of the air is effected by the retention of the air-borne particles in the filter. The degree of retention of particles depends on the number of contacts between the particle and the filter. The chance contacts, on the other hand, result from: (a) direct interception or trapping of the air-borne particles, (b) inertial impaction of the particles on the filter material, (c) air flow turbulence, (d) diffusion forces arising from Brownian movement, and (e) electrostatic attraction. Therefore, velocity, nature and size of the particles being filtered and the size and nature of the filter material all exert their independent influences (48).

Fibrous filters are simple in design and operation as well as free from operational hazards. Their disadvantages lie in the periodical checking and replacement required, and the rate at which they load with solid particles (74).

Ultraviolet Irradiation

Ultraviolet irradiation has been used to sterilize small volumes of air (75). A room air conditioner was equipped with ultraviolet lamps to purify the air for recirculation (76). It has also been used largely in reducing and controlling air-borne infections such as influenza, measles, and chicken pox in schools and in certain living quarters (77, 78, 79, 80, 81). Although some studies also report unsuccessful results in reducing air-borne infec-

tions in various areas.

The disadvantages of using ultraviolet irradiation for air disinfection are its cost, the danger it can cause to the skin and eyes, and the limited area it can sterilize, i.e., upper air in a room. Another serious disadvantage is that ultraviolet light will not penetrate a dust particle to kill organisms attached to the side opposite the light source.

Other Methods

Dry heat and wet heat (steam) have been used for air sterilization, but are either expensive or impractical. Electronic precipitators have been developed and show promise for use as air filters. The air is passed by charged plates having a potential of several thousand volts. The charged particles in the air are attracted to the oppositely-charged plates and are thus removed from the air. The plates may then be discharged, washed, and recharged for repeated use (74).

The Effect of Environmental Conditions on Air-borne Microorganisms

Microorganisms have been shown to grow in air by means of the "growing threads" method (82). The survival and subsequent infectivity of air-borne microbial particles are dependent on such factors as their source strength, particle size distribution, and environmental factors, i.e., water

content, relative humidity, temperature, sunlight, and ultraviolet light (83, 84). Numerous studies have been made to determine the survival of air-borne bacteria under different environmental conditions. The various test organisms used in these studies were aerosolized into test chambers under controlled conditions and the death rates determined (85). It is important to know that several factors affect the behavior of aerosolized bacteria (86). These are:

(a) the strain of a given species;
(b) the age of the culture;
(c) the metabolic state, as influenced by temperature;
(d) the chemical composition of the suspending medium;
(e) the nature of the material in the particle deposited by evaporation of the suspending medium.

Many workers have found that the stability of air-borne cells differs among genera. Therefore, in reviewing the studies made on this subject, the test organisms used were mentioned.

Water and Relative Humidity

The relationship between cellular water and the death of air-borne cells has been studied by Webb (87, 88). He suggested that the death of the cell resulted from the movement of water molecules in and out of the cell in an equilibrium system, resulting in collapse of the natural structure of cellular protein. An aged aerosol of Escherichia coli cells used in this study was killed immediately with rapid increases in relative humidity. Escherichia coli was also

studied by Poon (84), and Hayakawa and Poon (89). Both concluded that water evaporation from bacterial aerosols is the factor which governs the viability of bacteria. It was observed that the presence of saline water in bacterial aerosols increased the death rate of air-borne bacteria. The presence of a hygroscopic substance such as glycerol which reduced water evaporation, showed a considerable decrease in death rate. The death rate of E. coli was found to increase as the humidity approached saturation (90, 91, 92).

A study on washed, moist cells of Serratia marcescens, after storage in air at 20 to 30% water, showed a slight decrease in viability. But, at less than 1% water, the death rate was rapid (93).

An interesting experiment on the survival of some gram negative bacteria was done by McDade, et al. (94). Escherichia coli, Salmonella derby, Pseudomonas aeruginosa, Proteus vulgaris, and Proteus morganii were exposed on surfaces which included glass, ceramic tile and rubber tile. It was found that the survival of all the test strains used on all surfaces was best at 25°C and 11% relative humidity. Death of the organism progressed rapidly at 25°C and 53 or 85% relative humidity.

Salmonella pullorum dispersed from water into dust-free air streams showed an increased death rate when the temperature was raised from 28° to 37°C and as the relative humidity increased from 15 to 80% (95).

Several types of diphtheria strains and beta-hemolytic streptococci were allowed to dry in the dark on dust, textiles, earth, glass, paper, etc. They survived for quite a considerable time in the dried condition (96). This illustrated how pathogenic organisms could survive and be potential sources of infection for long periods of time.

Oral-pharyngeal secretions from patients with streptococcal infections were placed in sterile dust and dried for 4 to 8 hours. No appreciable decrease in the number of viable organisms was observed (97).

The death rate for Streptococcus pyogenes and Staph. aureus was found to increase more rapidly at higher relative humidities (92). The survival rate of staphylococci at different temperatures and relative humidities has been studied extensively (98). It was found that at 20°, 30°, 37°C, the survival of staphylococci was greatest at a relative humidity of 95 to 98%. There was an initial decrease in the number of viable cells but this was followed by multiplication of the staphylococci. On the other hand, at 25°C, survival was best at relative humidities of 11 and 33% (99). At 50°C, a 10% relative humidity was found to be optimum for the survival of some strains of Staph. aureus (100). Accelerated death rates were obtained in the above studies at temperatures of 20°, 25°, 30°, 37° and 50°C, when the humidity was greater than 50%.

Sunlight

Various studies to measure the lethal effect of sunlight on air-borne bacteria have been performed. An important finding was that daylight, on clear or cloudy days is a "potent" lethal agent for streptococci. An average of 50 min. exposure to sunlight was necessary to destroy 50% of an alpha-hemolytic strain of streptococcus. Beta-hemolytic strains were found to be less susceptible. The bactericidal effect of sunlight was found to be proportional to the intensity of light (101). In a dark room, the estimated time for 50% survival for Group A, beta-hemolytic and alpha-hemolytic strains of streptococci varied from 1 to 13 days (102).

Some workers considered the combined effects of relative humidity and light. Luckiesch, et al. (103) reported that E. coli was ten times more resistant to the action of 2537Å light in a thin layer of water than in air. Working with staphylococci and streptococci, Lidwell and Lowbury (92) found that at 60% relative humidity, exposure to daylight, fluorescent lights, or low intensity ultraviolet radiation increased the death rate of these organisms to approximately five times that obtained in the dark. The anti-bacterial action was less at lower humidities.

Ultraviolet Radiation

Ultraviolet radiation studies on air-borne organisms have shown that in relatively dry air, the lethal effect of

ultraviolet light was ten to 100 times greater than when organisms (E. coli) were irradiated on the surfaces of agar plates, in water or other denser media (104, 105). According to Webb, et al. (106), much of the damage caused by ultraviolet rays results from an alteration of the water molecules. They came to this conclusion after observing that the presence of bound water rendered the cells more susceptible to ultraviolet radiation, while its removal resulted in increased stability. They used E. coli, Ps. aeruginosa, and Ser. marcescens as test organisms. Similar conclusions were arrived at by other workers (107, 108), who used Pasturella tularensis, Bacillus anthracis, Coxiella burnetii and Brucella suis for their studies.

The Relation of Particle Size to Air-borne Infections

Air-borne infection has been defined as "the inhalation of droplet nuclei" (resulting from the evaporation of aerosol droplets) which remain suspended in air for relatively long periods of time (109, 110). That microorganisms commonly invade the atmosphere on dust particles, in droplets, or in the nuclei of instantaneously evaporated droplets was shown by Wells, et al. (111). The risk, therefore, of aerial spread of infection is proportional to the length of time the infectious particles are suspended in air. Particles dispersed in the size range of droplet nuclei would be more significant contributors to air-borne infection than larger dust-borne organisms since these settle out of the atmosphere more quick-

ly (109). Wells, et al. (112) showed that small particles, essentially single bacterial cells, could infect by inhalation, while larger particles containing viable cells failed to do so. This was confirmed by Lurie, et al. (113) who demonstrated that each infectious particle inhaled and retained in the alveoli produced a separate tubercle. Therefore, it was established that the infectious dose is a single organism in the proper site (110, 114).

Morton (115) proposed a set of postulates relating to the epidemiology of air-borne infection:

(a) the presence of air-borne viable infective organisms must be demonstrated;
(b) concentrations and particles must be measured;
(c) it must be demonstrated experimentally that these concentrations and particles can cause infections;
(d) it should be shown directly where the particles came from.

The size distribution of air-borne particles carrying microorganisms associated with human disease has been studied (116). Such particles were found to be in the range of 4 to 20 microns equivalent diameter[1]. Many fungi were found to be present in air as single spores. Noble (117) studied the size distribution of air-borne particles carrying Clostridium welchii. He found that whatever the size of sampling or the weather, at least 78% of particles carrying

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[1]The equivalent diameter of a particle is the diameter of a sphere of unit density which has the same settling-rate in still air (calculated on the basis of Stoke's law) as the particle in question (27).

Cl. welchii were more than 4.2 microns in equivalent diameter. Noble also observed that the large particles tended to disappear in wet weather but the smaller particles were fairly constant in concentration in wet and dry weather.

An interesting experiment was done by Lidwell, et al. (118). They collected samples of Staph. aureus from air. The form of the killing curve obtained by bombarding microorganisms with high energy electrons gave an estimate of about four viable cocci in each air-borne particle.

The port of entry and the site of deposition in the respiratory tract have received considerable attention by workers in the field of air-borne infection. Ehrlich (119) pointed out that the nose retains solid and liquid particles larger than 5 microns. Therefore, bacteria attached to particles of this size will be retained in the nose. Experiments to measure the per cent penetration of various particles through the human nose were carried out by Landahl and Tracewell (120). They obtained a 50% penetration for particle diameters ranging from 2.5 to 6.7 microns, using materials such as glycerol, methylene and others. In order to study the mechanism by which infectious materials can enter the body, traces of Bac. mycoides and bacteriophage T_3 were placed in the nose, conjunctiva, and mouth. It was found that traces placed in the nose passed rapidly down the throat and were present in small amounts in the saliva. The organisms were dispersed by blowing the nose and more efficiently by sneezing (121).

Ostrom, et al. (122) exposed human subjects to an aerosol of Ser. marcescens for 5 min. The oropharyngeal region was then sampled by a combination mouthwash-gargle technique within 2 to 5 min. after exposure. They found a correlation between the percentage recovery and the number of bacteria inhaled (dose). After exposing subjects to bacterial aerosol and sampling at intervals up to 2 hours, James, et al. (123) came to a similar conclusion. James, et al. (124) and Meyers, et al. (125) also concluded that the shorter the delay before sampling, the better the recovery probability.

The size range for the greatest alveolar deposition was found to be around 1 to 2 microns (126). For finer particles, alveolar deposition decreased to a minimum at about 0.25 micron and then increased again with further decrease in size, as the magnitude of the Brownian movement increases.

Some Occurrences of Air-borne Infection

Numerous studies incriminating air-borne mode of infection have been reported. Air-borne infection in the laboratory has been one of the subjects of study (127). Johansson and Ferris (128) have shown by high speed photography and air sampling techniques that laboratory operations such as pipetting, pouring and vigorous agitation of dilution blanks often produced bacterial contamination of the surrounding air and environment. This shows the apparent danger in highly infectious agents. Infection by inhalation of microbial aerosols was shown to be a major hazard in

research laboratories. Certain procedures involving highly infectious materials such as grinding of tissues in a blender, concentrating in a centrifuge and others are highly dangerous (129).

Dust has been studied as a vehicle for the spread of infection (92). Large numbers of hemolytic streptococci, staphylococci, pneumococci, diphtheria bacilli and tubercle bacilli were demonstrated in the floor dust of hospital wards (92, 129). Dust artificially infected with hemolytic streptococci was infective for mice at the end of 10 wk. A few viable streptococci in dust were viable 195 days after inoculation (130). Corynebacterium diphtheriae, an air-borne infective agent, remained viable in dust for 102 days (131, 132, 133).

The seasonal pattern of measles and chicken pox was shown to be transmitted by air-borne droplet nuclei (9). An outbreak of 128 cases of brucellosis occurred in a large slaughter house in Iowa employing more than 1000 workers. The highest attack rates occurred among those stationed in the kill rooms. These people had close contact with fresh tissues and tissue fluids of the animals. The authors concluded that the circumstances of this outbreak strongly point to air-borne infection (110, 134).

The first reported outbreak of inhalation anthrax occured in New Hampshire in 1957 (135). Five cases were found, four of which were fatal. The incident took place in a goat-hair processing mill. Those affected had the dustiest

occupation in the mill. The air was found to be highly contaminated with anthrax spores. Many particles were below 5 microns in size and this indicated the air-borne nature of the outbreak.

An infection of Q fever involving 75 cases broke out in Oakland and neighboring cities in 1959. Almost all of the victims lived in a narrow triangular path, seven miles long and one mile wide. The few cases who resided outside the path had regular employment or frequent visits to places within the path. At the point of the triangular path was a rendering plant for sheep and goats. Observations showed that the direction of the path was in line with almost constant prevailing winds. Thus, the rendering plant was incriminated as the source of air-borne infection (110, 136).

Two poultry-dressing plants were the site of five outbreaks of psittacosis in which 96 cases and 7 deaths occurred. The highest attack rates of over 50% occurred among the workers who killed the birds and picked the pinfeathers. The lowest attack rates were among the graders. Packers in a separate room escaped infection completely. It was difficult to differentiate between contact and air-borne infection in this type of processing. But the high attack rates and the explosive nature of the outbreaks led the investigators to conclude that air-borne infection played a major role (137).

An increasing amount of circumstantial evidence suggests that salmonellosis may sometimes be transmitted into man by air-borne means. Darlow, et al. (138) experimented with mice infected with an aerosol of S. typhimurium. The lethal dose was much smaller than that required by ingestion. On the other hand, Clemmer, et al. (139) used chicks in their study. They showed that chicks can be infected with S. pullorum by the respiratory route. The inhaled organism multiplied to a significant extent in the lungs over a period of several days.

Salmonellae were found in the nose, sputum and throat of children suffering from salmonellosis. Therefore, the disease possibly may be air-borne (140). Other cases of salmonellosis where the organism was isolated from ward dust, hospital linen, and environment were reported by several investigators (141, 142, 143).

Environmental Studies in Various Premises

Public health history has shown man's attempt to protect himself against any part of his environment that will prove antagonistic to his well being (144). Environmental microbiology was defined by Greene (1) as "the systematic quantitative and qualitative study of microorganisms in man's physical environment, the effect of the environment on the organisms, and the effect of the environment in the transmission of the infectious disease agents". The microbial contamination load in air is a function of three variables

which must be controlled to minimize air-borne contamination (145). These are: (a) the rate at which the contamination is being introduced into the air from people and surfaces, (b) the rate at which the contaminated air is being replaced with new air, and (c) the purity of the clean air being introduced.

A. Studies on Air-borne Microorganism Populations

Occupied Places

Earlier studies on the air of occupied places, i.e., subways, theaters, streets and parks, enumerated total air-borne bacterial counts. Str. salivarius was used as an index of recent air pollution from the mouth and respiratory sources (146, 147). The concentration of Str. salivarius in the air of schoolrooms and lecture rooms rose with an increase in the amount of talking and with activity, to a lesser extent (148, 149, 150).

Hospitals

A study of the bacterial populations in the air of operating theaters showed an average range of 15 to 107 bacteria-carrying particles in 30 liters of air. The total number of bacteria was influenced by the type of activity and the number of people present in the theater. An increase in the number of people increased the Str. salivarius count. On the other hand, preparation for operation, dusting and cleaning increased the total count and the "Bacterium coli" count. The air of infant wards was found to be highly con-

taminated with "Bact. coli". This was due to poor nursing technique and was suspected to be related to an epidemic of infant diarrhea that occurred (149).

The levels of air-borne contamination in various areas of two hospitals were determined by Greene, et al. (151). They obtained a mean count of 20 colonies per cu. ft. for the entire hospital environment with 48% associated with particles greater than 5 microns diameter, 30% with particles between 2 and 6 microns diameter, and 22% with particles less than 2 microns diameter. It was concluded that the air-borne contamination was influenced by traffic, activity, ventilation, gross surface contamination but not by seasonal changes.

Characterization of the isolates from the above study showed that 42.6% were gram positive cocci, 19.2% were gram positive rods, 14.0% were gram negative rods, 17.1% were molds, 2.2% were actinomycetes, 1.2% were yeasts and the remaining were diphtheroids and cocco-bacillary type organisms. The authors found that the distribution of types varied according to hospital area, locations within a given area, and a level of gross air-borne contamination, but did not vary significantly with the season of the year. A relationship between contaminant particle size and type of organism associated with the particle was apparent (152).

The fungal flora of the air of hospital wards (particularly those that grow at 37°C.), was investigated by Noble and Clayton (153). Aspergillus fumigatus was the main

species isolated and recovery of this mold was highest during the autumn and winter months. It was found on blankets in the wards.

Other organisms studied with interest in relation to air-borne hospital infections were coagulase positive staphylococci, hemolytic streptococci and Cl. welchii (154, 155, 156, 157).

Industrial Clean Rooms

An interesting study comparing industrial clean rooms against hospital operating rooms was done by Michaelsen and Vesley (158). They found that on the average, the air-borne microbiological contamination level in industrial clean rooms was about one-third that found in hospital operating rooms studied. This was explained in terms of the total activity which was greater per unit area in operating rooms than in industrial clean rooms.

Portner, et al. (159) studied the level of aerobic aerial contamination in a clean room occupied by personnel wearing clean room clothing, street clothes and either sitting or active. Very little microbial contamination in the air was observed when the clean room was vacant. But when the personnel came in, the microbial contamination increased greatly. This was apparent, even when the personnel wore surgical masks and clean room clothing. Personnel movement and the wearing of street clothes increased the microbial count. Immediately after the personnel left the clean room

the level of microbial contamination decreased.

The predominant types of bacterial contamination found in conventional industrial clean rooms and in laminar air flow clean rooms were determined by Favero, et al. (160). In the horizontal laminar flow clean room, no viable particles were detected at the filter wall. But as the air moved past personnel toward the exhaust wall, the level of microbial contamination increased progressively. When the personnel left the room, the number of viable particles decreased. The majority of microorganisms isolated from the bench top surfaces by the Rodac plate method were staphylococci species, micrococci species, and the corynebacterium-brevibacterium groups. Few sporeformers, molds and actinomycetes were detected. The prevalent types of organisms isolated were indigenous to human skin, hair and respiratory tract.

Hatcheries and Poultry Processing Plants

Large populations of air-borne bacteria were detected in several hatcheries. The air-borne counts varied from hatchery to hatchery but were not influenced by the season of the year or the makes of incubators (setters) and hatchers. The magnitude of the microbial counts was regarded as an indication of the sanitary status of the operation (161).

The Andersen sampler was used by Gentry, et al. (162) to evaluate the sanitation of some hatcheries. They found that hatcheries with poor floor designs and faulty traffic

patterns were highly contaminated compared with those of one-way traffic flow. Chick processing and washing rooms were also found to be highly contaminated.

Air-borne microbial counts were influenced markedly by the stage of hatching, employee activity, location of the site examined, and residual microbial contamination (163). In a later study (164), Magwood and Marr observed that air-borne bacterial counts were proportional to those on horizontal surfaces such as floors and table tops. They speculated that the organisms become air-borne from employee activity and are drawn into the hatchers where they multiplied during hatching. These were expelled from the hatching machines on dust and fluff and re-settled on the horizontal surfaces. This cycle would be repeated during successive hatches.

The effect of small and large air-borne bacterial populations on the degree of contamination of eggs, shell membranes and other surfaces were studied during incubation and hatching (165). A direct relationship was observed between the air-borne population and the degree of contamination of the various surfaces. In clean air, the bacterial counts of egg shells dropped quickly and low counts persisted on all surfaces to the completion of hatching. On the contrary, if high counts were found on the egg shells, the counts decreased progressively until low counts prevailed up to the completion of hatching. The author concluded that in order

to minimize bacterial contamination of eggs and hatching birds, hatchery premises should be kept free of reservoirs of contamination which become air-borne readily.

A study was made to determine whether the rendering process of diseased turkey tissue in a turkey plant caused aerosols of infective organisms (166). High counts were found in the area of the grinder and blow tank which confirmed the production of infectious aerosol during the rendering process.

Concentrations of total aerobic bacteria, molds, yeasts, coliforms, enterococci and psychrophiles were determined in the air of two poultry processing plants, during plant operations. Total counts were highest in the dressing room, with decreasing numbers in the shackling, eviscerating and holding rooms. The average counts per cu. ft. of air in these areas were 2200, 500, 230 and 62, respectively (167).

Dairy Plants

One of the earliest works reported on air-borne microorganism populations in dairy plants was by Olson and Hammer (168). The number of microorganisms, yeasts, and molds falling on exposed agar surfaces were determined. They found bacteria to be more numerous than yeasts. The number of organisms collected indoors and outdoors did not differ significantly. No distinct seasonal variation was observed. That more bacteria than yeasts were falling on exposed agar plates was also observed by the same authors who collected

samples inside and outside butter churns. They found that molds were present in larger quantities than yeasts (169).

The number of air-borne bacteria, yeasts and molds in various areas in the dairy plant was determined by Heldman, et al. (170). The mean air-borne bacterial count for all areas investigated was below 6 per cu. ft., 2 per cu. ft. of yeasts and 12 per cu. ft. of molds. Significant day to day variations in all areas were observed. Some degree of correlation between worker activity and air-borne counts were noted.

A study of the same dairy plant areas was performed by Sunga, et al. (171). A mean air-borne bacterial count of 58 per 5 cu. ft. was obtained as compared to 27 per 5 cu. ft. in the previous study (170). The increase in counts was attributed to the building construction adjacent to the dairy plant.

The effect of flooding floor drains on the air-borne microbial populations in a dairy plant has been investigated (172). It was shown that bacterial counts obtained during flooding were 140 per cu. ft. where normal counts were less than 10 per cu. ft.

A flooding wash bowl in a dairy plant was found to act as a source of air-borne microorganisms, in a manner similar to flooding floor drains. A ventilation system was found as a consistent source of air-borne microorganisms, with increased contribution caused by periodic stopping and starting of the system (173).

Cannon (174) obtained mean counts of 92.3 per cu. ft. of non-mold colonies and 51.3 per cu. ft. of mold colonies from ten fluid milk plants studied. A wide variation in populations was observed.

A study of the sanitary significance of air in relation to ice cream was performed by Fabian (175). His results showed that considerable numbers of bacteria and molds were present in the air particularly during April and less in January. He concluded that the weather was the most important factor in determining the number of microorganisms in the air. A similar conclusion was arrived at by Macy, et al. (176) who observed the quantitative changes in the air microflora during the manufacture and storage of butter. They obtained high counts of molds and yeasts in their 10-min. exposure of plates during the summer.

Air samples from cheesemaking rooms at seven creameries were examined for lactobacilli. Colony counts ranged from less than 1 per cu. ft. to greater than 65 per cu. ft. The most commonly isolated species were Lactobacillus casei, L. plantarum, and L. brevis (177).

B. Some Measures of Control Advocated Against Air-borne Contamination

Hospitals

Several recommendations have been given by various workers on methods of controlling air-borne bacteria in hospitals with special reference to operating rooms and patient

areas. By the application of air engineering, air that is sterile in surgery rooms, dust-free and odor-free can be provided for hospitals.

Floor sweeping and furniture dusting are best done with central vacuum cleaning systems (178). This allows the blower, dust separator and discharge dust to be located out-of-doors, thereby, eliminating air-borne contamination indoors. The same author emphasized the importance of hospital fresh air intake locations in order to avoid the introduction of contaminated air from the power plant, laundry, incinerator, kitchens, laboratories and toilets.

According to Avery (179), hospital air conditioning particularly for operating rooms is either a process or comfort air conditioning. It is comfort conditioning when it provides a suitable environment in which the surgeon and his crew perform their work. On the other hand, it is process ventilation when filtered air that becomes sterile is supplied with a flow pattern that passes over the operation site and prevents infection of the open wound. The suggestion for contamination control was to fill the entire ceiling of the operating room with high efficiency particulate (HEPA) filters[2].

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[2] These filters have an efficiency of 99.97% and remove air-borne droplet nuclei and dust-borne bacteria down to a diameter of 0.3 micron.

The reduction in the number of air-borne bacteria through a HEPA filter depends on the number of air changes. Presently, the rate of air circulation required in operating rooms by federal and state laws is "eight air changes per hour". Cole, et al. (180) reported that this was inefficient in the removal of air-borne bacteria. They recommended a range of 20 to 40 room air changes per hour for an 8 x 8 x 8 ft. operating room model.

It has been common practice in hospitals to wear special caps and gowns made from cotton. Unfortunately, cotton sheds lint readily but it can be sterilized, thereby eliminating a bacterial source. Masks are required in critical areas in a hospital. Droplets of material expelled from the nose and mouth while speaking, laughing, coughing and sneezing are eliminated from becoming air-borne (158) by using the proper mask.

The cleanliness of a hospital operating room is based on the bacterial content of the air. Standards of air cleanliness for hospital operating rooms have not been established. Among the few suggestions are 1.0 organism per 10 cu. ft., 1-2 organisms per cu. ft. and 5-10 organisms per cu. ft. (158).

Industrial Clean Rooms

Federal Standard No. 209 "establishes standard classes of environmental air control within clean rooms and work stations". Its objective is "to prescribe air cleanliness

classes and other environmental conditions (See Appendix 4) required for achieving and maintaining levels of cleanliness specified in the product specification" (181).

A recent method of improving environmental control in clean rooms has been through the use of laminar air flow (182, 183, 184). Among the different types of laminar air flow are the following (181):

(a) wall-to-wall laminar flow - air enters the room in a laminar pattern through HEPA filters set in the wall over the work bench and leaves the room through a grated floor. There is a pre-filter in the floor exhaust plenum ahead of the blower;
(b) ceiling-to-floor laminar flow - air enters through a HEPA filter, flows downward and leaves the air room through a grated floor;
(c) wall-to-wall laminar flow - air enters through one wall and leaves through the opposite wall through a grill and pre-filter. Small bench units for laboratory space, which are low in cost and easy to maintain have been designed.

Laminar air flow uses highly filtered air moving in layers, thereby creating a sterile pressure gradient against the intrusion of contaminants. Laminar pattern is such that the entire volume of air within the area moves with uniform velocity along parallel flow lines. There are no eddies or dead areas in this stream. Therefore, particles cannot settle out in the region at random (185).

The 17th edition of the Pharmacopeia of the United States of America (186) recommended the following measures to minimize air-borne microbial contamination in aseptic filling areas:

(a) employ air filters, electrostatic precipitators, banks of ultraviolet lights or combinations of these devices in the air systems supplying these areas;
(b) provide air pressure that is slightly positive to that in adjacent areas;
(c) protect dust-free rooms from sudden changes in pressure and air movements by providing anterooms or air locks at all entrances;
(d) sterile caps, masks, gowns, rubber gloves and shoe covers must be used by the personnel;
(e) traffic should be minimized and visitors not permitted in areas where aseptic filling is conducted;
(f) carry out aseptic operation in specially constructed sterile hoods, whenever the nature of the operation permits it.

Food Processing Areas

Several suggestions for the control of microorganisms in food plant air have been enumerated by Hedrick, et al. (187): (a) location of the plant in an area with low outdoor ambient levels, (b) sanitary plant, (c) minimum number of workers, (d) sanitization of drain traps, (e) clean storage rooms, (f) maintenance of good ventilation systems and (g) a minimum product exposure time to the air.

The recent use of flash pasteurization for beer has resulted in an air sanitation problem for the brewing industry. Aseptic canning or bottling was used after pasteurization which needed to be done in a sterile atmosphere (179).

Sterile air, forced through the mash, has been used in the fermentation of various products. This prevented the introduction of wild yeasts, at the same time provided oxygen for biochemical processes and gave a better yield (179).

In the carbonated beverage industry, sterile air is also a vital commodity. Filtered air has been used to replace the volume of the drained syrup at the top of tanks. This prevents scum formation and subsequent spoilage of the syrup (179).

Langmuir (188) recommended that the first step in preventing the occasional, sharp outbreak of psittacosis in poultry processing plants should be the development of better facilities for worker protection. Certain specific processes on the slaughtering-dressing line may be responsible. Therefore, such processes might be changed or hoods and controlled air currents used to keep the infection from the workers.

III. Humans As Sources Of Air-borne Microorganisms

The Skin as a Habitat for Microorganisms

A. The General Anatomy of the Skin

The skin constitutes 10 per cent of the total body weight. In an average adult, it has an area of approximately 1.8 square meters. According to Leider and Buncke (189), the surface area of the skin in different areas of the body, expressed as a percentage of the total area are:

Head	9%
Arms	18%
Chest and back	36%
Perineum	1%
Legs	36%

The skin consists of two layers, the epidermis and the dermis. The epidermis is on the surface and is composed of cells in varying stages of keratinization. The topmost layer of the epidermis is the stratum corneum. It consists of several layers of flattened dead cells which are firmly attached to each other. These layers contain homogenous horny masses of keratin. The outermost layer of the stratum corneum is constantly shed as minute particles. The loss is replaced from the layers below. The epidermis, therefore, is made up of cells consistently moving upward towards the surface. The cells are in successive stages of differentiation and decay, until they are finally dissipated to the environment. This process takes part in providing a constant replacement of nutrients for microorganisms living on the surface of the skin. There are no blood vessels in the epidermis and nutrients and oxygen for epidermal cells diffuse out from the blood vessels by permeation (190).

The dermis is underneath the epidermis. It is a connective tissue layer which contains blood and lymphatic vessels. It provides the cells of the skin with their metabolic requirements - oxygen, nutrients and structures for the defense mechanism of the body. In the skin, the blood stream functions as a regulator of body temperature. Microorganisms inhabiting the skin would depend on the blood stream for their nutrients. These organisms would be sub-

jected to variations in temperature depending on the internal condition of the host and other external factors. More nutrients are available to the microorganisms in the dermis and one would, therefore, expect more fastidious organisms to thrive on this part of the skin.

From the epidermis growing down into the dermis are the cutaneous appendages or the sweat glands which provide the protecting and supporting connective tissue sheaths. There are two kinds of sweat glands, the eccrine and the apocrine. Eccrine sweat glands are found only in primates. These glands are found all over the body, but their density varies from region to region. The apocrine sweat glands have a more limited distribution especially in man. They are most important in other mammals (190).

The eccrine glands consist of three distinct parts: (a) the proximal part lying deep in the dermis which is coiled, (b) this leads to a straight tube that goes through the epidermis, and (c) the epidermal segment of the sweat duct is again coiled which leads out of the secretory tubule through an ampula. The eccrine glands are the constant suppliers of nutrient to the skin surface. Water is the most important substance that these glands secrete. Their secretion aids in preserving the "acid mantle" of the skin which prevents colonization by pathogenic organisms. Another important function of the sweat glands is the provision of a mechanism for the disposal of excess body heat.

This results in the maintenance of a steady temperature.

The regional distribution of the sweat glands on the body is important because this contributes to the differences in the climate of the skin. The areas of the skin carrying eccrine sweat glands provide special conditions of habitat for microorganisms. The eccrine glands are most numerous on the palms and finger pads, soles, head and forehead.

The important structural features of the skin are the folds and ridges, the distribution of cutaneous appendages, the presence of keratinized and desquamated cells, and the changes in the features during the life of the host. It is these features which determine the supply of nutrients to microorganisms and the micro-climates in which they live (190).

B. Ambient Conditions on the Skin

Temperature

There is a great deal of variation in the temperature of the skin, depending on factors such as changes in atmospheric conditions, air temperature, relative humidity, amount of clothing and site of the body. The temperature of the skin is only rarely and for short periods raised above 37°C. In a regular comfortable environment, the general level is maintained between 30° and 33°C. or about 5° below the internal temperature (191).

pH

Rothman and Lorinez (192) reported that the skin surface has an acid pH range which is bacteriostatic and is called the "acid mantle". Bacterial species display different sensitivity to pH. "In general, streptococci are more sensitive to acid milieu between pH 3 and 5 than are staphylococci" (192). The skin has the ability to restore its acidity, if contact with alkali occurs. The acid pH and the buffering capacity are brought about by substances from the sweat glands. These are the lactic acid-lactate buffer system and the amino acids.

The pH of the skin on the forearms of adult males and females, and children was measured by Blank (193, 194, 195, 196). He found the majority of the readings within the range of pH 4.2 to 5.6.

Humidity

The surface of the skin is kept in a moist condition by: (a) the continuous supply of water from the deeper tissues by means of transepidermal permeation and sweat gland activity; (b) a continuous but fluctuating loss of water by evaporation; and (c) the holding of water in the layers of the epidermis by substances some of which are produced as a result of keratinization (190).

Evaporation is controlled by: (a) degree of occlusion by opposing skin surfaces or restrictive clothing; (b) wind velocity; (c) relative humidity and (d) temperature

of the surrounding air. In a still atmosphere, the surface of the body is covered with zone of air, approximately 6 ml. in depth, which is saturated with water vapor (190). Therefore, the areas of the body most susceptible to microbial infection are those protected from the evaporative and cooling effects of moving air. Loss of water by evaporation results in a further decrease in the pH of the skin surface thereby, encouraging bacteriostatic conditions.

The output of sweat varies in the different areas of the body in response to different internal and external stimuli. Increased sweating or moisture content of the skin produces an increase in the number of microorganisms present (197, 198, 199). Fluctuation in the output of the eccrine sweat glands could have a great effect on the skin's surface flora (200).

C. Nutrients on the Surface of the Skin

The nature and availability of the various nutrients to the microorganisms living on the skin will determine the presence and multiplication of these microorganisms on the skin. Active secretion of the eccrine sweat glands brings fresh supplies of moisture and dissolved nutrients to the skin surface for the nourishment of the microbial flora.

Nitrogenous Compounds

Nitrogenous compounds have been found to be present in appreciable quantities on the surface of the skin. The microbial population of the skin would, therefore, have an

available source of nitrogen for metabolic purposes. Mitchell and Hamilton (201) have found that the amount of nitrogenous compounds present depends on the sweating rate. They recorded the following figures for adult males:

in "hot-humid" environment	22.7 to 26.6 mg./100 ml.
in comfortable conditions	16.5 to 17.4 mg./100 ml.

Most of the nitrogen available on the surface of the skin is contained in urea, amino acids, and other nitrogenous substances such as uric acid, creatinine, creatine, ammonia and choline.

According to Rothman (191) recent investigators have obtained figures between 30 to 60 mg. of urea N in 100 ml. of sweat. He quoted an average of 44.9 mg. urea/100 ml., which was the result obtained by Gad-Andersen in his study in 1926. The concentration of urea in sweat was higher than in blood serum (202).

Amino acids reach the surface of the skin by trans-epidermal diffusion and secretion of the eccrine sweat glands. Various investigators have detected up to 20 free amino acids present on the surface of the skin (203, 204). A list of these amino acids is given in Table 1. With this varied and constant supply of amino acids, the skin's microbial population should have at least the minimum requirements for growth. The limited number of species living on the skin would not be due to lack of essential nitrogenous nutrients but to other environmental factors (190).

TABLE 1.--Free amino acids detected on the skin surface

Amino acid	Investigator: Burke, et al. 1966 (203)	Investigator: Hamilton 1965 (204)
1. Taurine	+	+
2. Aspartic acid	+	+
3. Threonine	+	+
4. Serine	+	+
5. Glutamic acid	+	+
6. Proline	+	+
7. Citrulline	+	+
8. Glycine	+	+
9. Alanine	+	+
10. Valine	+	+
11. Cystine	+	+
12. Methionine	+	+
13. Iso-leucine	+	+
14. Leucine	+	+
15. Tyrosine	+	+
16. Phenylalanine	+	+
17. Ornithine	+	+
18. Lysine	+	+
19. Histidine	+	+
20. Arginine	+	+
21. n-amino-n-butyric acid		+
22. Tryptophan	+	
23. Cysteine	+	
Other nitrogenous compounds		
24. Urea	+	+
25. Ammonia	+	+

Electrolytes

1. Salt

Salt is always present on the surface of the skin. Rothman (191) calculated that an average of 128.7 mg. of salt is deposited per square meter of the skin surface in 24 hr. The quantity of salt that reaches the skin surface increases with sweating (205). Therefore, upon evaporation, the salt concentration on the skin becomes considerable. It follows that only those organisms that can withstand considerable salt concentration (like Staph. aureus) will be able to colonize the skin surface. Washing or bathing removes the salinity of the skin surface but it is immediately supplied from below. Compared to plasma, sweat is a hypotonic solution containing concentrations varying from 0.29 to 0.50% which is approximately half that of blood plasma. The concentration varied with the individual and with time. The climate where the individual resided also affected the salinity of the skin according to Conn (206).

2. Others

Potassium is present in the aqueous film with sodium (207). In the absence of visible sweating, the ratio of K:Na is approximately 1:1. During active sweating, the ratio falls slowly to 1:9. The concentration of potassium on the skin surface is similar to its concentration in the blood (191).

Calcium is present in sweat in concentrations from 2 to 7 mg./100 ml. (190). It is found in decreasing concentrations as sweating progresses. Magnesium, phosphorus, and traces of copper and manganese have been found in sweat (201). Zinc has also been observed in traces and is believed to be in the aqueous phase (208). Results of studies concerning the amount of iron present in sweat have been varied (209). Hamilton (204) recorded 0.1 to 0.2 mg. of iron in 100 ml. of sweat and calculated that 6.5 mg. were carried to the skin surface in 24 hr.

Lactic Acid and Lactates

Values for the concentration of lactic acid in sweat have varied from 73 to 350 mg./100 ml. (190). Rothman (191) believed that lactic acid is produced from glycogen in the sweat glands via the anaerobic, glycolytic reaction. Lactic acid is believed to be present in sweat as free acid and as lactate. These substances probably play a role in the "acid mantle" and buffering capacity of the skin.

Glucose

Glucose has been found in minute quantities on the surface of the skin. An average value of 3.08 mg. glucose per 100 ml. in profuse sweat and 2.86 mg./100 ml. in intermittent sweat were obtained by Lobitz and Osterberg (210). Due to the meager amounts of glucose present, its role as an energy source for the microorganisms on the skin surface has been questioned.

Lipid Substances

The lipid substances found in the skin are distributed over the surface as a fine emulsion in the aqueous film. The main sources of this emulsion are the sebaceous glands, desquamated skin and secretions of the apocrine sweat glands in areas where they are present. The lipid constituents that have been found are fatty acids, triglycerides, cholesterol, squalene and other hydrocarbons. The extent to which microorganisms utilize lipid substances as a nutrient has not been assessed. There has been evidence that Pityrosporum ovale can utilize sebaceous materials (190). On the other hand, some lipid substances like the fatty acids have been found to be both fungistatic and bacteriostatic (211, 212).

Vitamins

In general, the skin contains small amounts of vitamins. This may be a limiting factor for the microbial population of the skin. Rothman (191) reported the presence of several B complex vitamins in sweat, namely: niacin, pyridoxine, folic acid, pantothenic acid, biotin, inositol, and para-amino-benzoic acid. Thiamine and riboflavin were either present in trace amounts or were absent. Several of these B complex vitamins increased in concentration with increased sweating.

Other vitamins that have been found on the skin surface are ascorbic acid, largely in the epidermis and vitamin E.

D. Normal Skin Bacteria

Price (213) classified the flora of the skin into two groups, the transient and the resident flora. The transient flora includes any bacteria which are deposited on the skin from the environment. The resident flora includes the stable bacterial population of the skin that lives and multiplies in the skin layers, hair follicles as well as on the surface of the skin.

The transient flora that contaminates the skin include Ps. pyocyanea, Ps. aeruginosa, strains of salmonella, shigella, E. coli, Aerobacter-Klebsiella species, Alcaligenes faecalis, Staph. aureus, hemolytic streptococci, Str. viridans, sarcina, Pr. vulgaris, Chromatium minutissimum, aerobic spore-formers, Herellea vaginicola, Mima polymorpha (214, 215, 216). Transient organisms may remain viable for varying periods of time or they may multiply on the skin surface, depending on local environmental conditions.

The resident flora are mostly non-pathogenic commensals, but Staph. aureus may sometimes be included (217, 218, 219). Kligman (220) gave an outline of the organisms one may expect to encounter frequently or rarely on the normal skin. The major inhabitants of the skin are Staph. albus, Staph. epidermidis, corynebacterium or diphtheroids which are aerobic. Anaerobic corynebacteria are also found in numerous numbers, i.e., C. acnes. Those organisms found in small numbers are micrococci, Staph. aureus, streptococci and

aerobacter. Dineen and Hildick-Smith (216) have enumerated about 12 species of fungi that have been isolated from the normal skin of the hand.

A study of the bacterial population of the skin by Ulrich (221, 222) showed that there is a relatively consistent pattern of distribution among individuals. The head, axilla, perineum, hands and feet supported a large bacterial population. Other areas carried low or moderate numbers. Each person maintained a stable level of bacteria within a limited range whether low, intermediate, or high. There was a moderate day-to-day fluctuation. But climatic changes and sex caused no apparent variations.

Lovell (223) discovered that the sebaceous glands are the source of the resident flora. He removed sections of the skin, incubated them for 6 hr. in a moist chamber to permit the number of bacteria to increase and studied them microscopically. Higher bacterial counts were observed in areas well supplied with sebaceous glands (217).

Several investigators have published figures of the number of bacteria isolated from the skin surface:

(a) 6 to 865,000 per sq. cm. Evans, et al. (197)
(b) 1 to 1 million per sq. cm. Updegraff (224)

According to Evans, et al. (197), the anaerobes outnumbered the aerobes 10 to 100-fold in most cases.

Numerous methods have been designed to isolate bacteria from the skin (225). These are:

(a) culturing the skin surface or imprinting the skin on a nutrient medium (226);
(b) serial-basin surgical scrub technique, or the Price technique and its modifications (216);
(c) the use of rotary brushes (216);
(d) culture of excised skin (216);
(e) skin-stripping methods (224, 226).

Studies on the Shedding of Micro-organisms by Humans

A. Quantitative Measurements of Micro-organisms Shed by Humans

A relationship was found by early workers between air-borne infection and activities that increased air-borne particles such as sweeping, disturbance of bedding and bodily movement. Duguid and Wallace (227) measured the number of bacteria-carrying dust particles liberated into the air from a person's skin and clothing. The number of bacteria-carrying dust particles liberated was proportional to the amount of activity done by the person. They also showed that air contamination with dust-borne bacteria from clothing was reduced to half when a sterile loose cotton gown was worn over the ordinary clothing. But when a sterile dust-proof gown was worn, the reduction was down to a tenth or a twentieth.

Several other workers observed that air counts of staphylococci increased either with the disturbance of beddings and clothes or increased bodily movement. According to Bethune, et al. (228), the number of Staph. aureus in the area of occupied rooms varied widely but was usually 1 per 100 cu. ft. They defined a "dispenser" as one who,

while in the test chamber gave a Staph. aureus air count of 10 or more per 100 cu. ft. The dispersal of bacteria from patients was reduced by enclosing them in disposable paper instead of cotton sheets. Male subjects yielded about twice as many organisms as female subjects. Noble (229) found that the difference in dissemination between males and females was due largely to the difference in clothing.

The ability to disperse staphylococci depended on the degree of contamination of the skin with the organism (230). Solberg (231) confirmed that the difference in the ability of nasal carriers to disperse staphylococci into the air depended mainly on the number of organisms in the nose and skin. He suggested that the estimation of microorganisms on the fingers and hands was the best basis for determining whether a nasal carrier was a "heavy dispenser" or not.

According to Rothman (191), a complete layer of skin is desquamated every one or two days. Results from the study of Davis and Noble (232) suggested strongly that desquamation plays a major part in the dispersal of bacteria from the skin surface. The particles of skin had a mean equivalent diameter of approximately 8 microns. Cocci, in chains and singly as well as occassional bacilli have been seen growing on skin scales (233). Bacterial species carried on skin scales were identified and found to be mostly cocci

As the level of personnel activity increased, so did the air-borne microbial contamination in industrial clean rooms (239). This study showed that the chief sources of microbial contamination were associated with the personnel. This was supported by the fact that the total number of air-borne viable particles increased or decreased depending on the presence of personnel in the area. Furthermore, most of the microorganisms isolated from the air and surfaces were associated with the skin, nose, and mouth of humans.

Considerable amounts of contamination in clean rooms are introduced by humans (240). They track dirt or shed organisms through breathing or by the abrasive effect of clothing rubbing on the skin. This contamination is dispersed in the air and settles on horizontal surfaces. The floor, having the largest surface area catches and retains an enormous amount of contamination.

Austin (241) drew typical curves showing the fluctuations in air-borne contamination in industrial clean rooms. The increased personnel activity, upon their entrances and exits to the clean room, always resulted in an increase in air-borne bacterial counts. A very large increase in the contamination level always occurred after a break period. The author gave the following reasons for this: (a) personnel have spent time in highly contaminated areas and picked up contaminants on their person and clothes.

(b) some personnel have smoked and the tobacco smoke exhaled from their lungs contribute to the contamination level of the room, (c) food particles are being thrown off from the mouth, nose and lungs of personnel who had eaten.

The micro-biotank, a 7 feet high and 3 feet diameter chamber made of stainless steel was used by Reimensnider (242). The chamber was sterilized by steam and cooled by filtered air. The microorganisms shed by the subjects were recovered by rinsing the chamber with sterile 0.25% chilled peptone water. This was collected at the bottom of the tank and transferred into a sterile container for analysis. After serial dilutions were plated out in trypticase soy agar (after centrifuging the original aliquot), plates were incubated at 37°C. for 48 hr. Results showed that there was very little difference between daily runs for each individual when they wore a sterile scrub suit, socks and cap. The counts obtained when a germicidal detergent was used for showering were somewhat lower than those obtained with conventional soap. The investigator concluded that good personal hygiene and the wearing of sterile clothes reduced the number of microorganisms shed into the environment.

Qualitative determinations were performed on the microorganisms shed by subjects in the micro-biotank (243). A majority of the microorganisms were Staph. aureus, Staph. epidermidis, bacillus species and corynebacterium species. Spore-forming, gram positive rods were rarely isolated.

More recent workers on the shedding of microorganisms by humans have used a sealed plastic tent in which the subject stayed and exercised from 5 min. to 1 hr. Samples of air were then drawn and enumerated (244).

B. Suggested Measures to Control Microorganism Shedding by Humans

Bacterial contamination of air was very much less in tests with dust-proof gowns than in tests without gowns (227). Bernard, et al. (245) found that wearing suits of an impermeable substance such as polyethylene or a closely woven fabric with an average pore size of 10 microns virtually eliminated the shedding of skin bacteria to the environment. They also recommended that women wear trousers which were occluded at the ankles rather than skirts for operating-room clothing. A similar recommendation for men was given by Blowers and McCluskey (246). These same authors found that closure of neck and arm openings and wearing a one-piece suit caused no reduction in bacterial dissemination.

Masking has also been suggested as a measure against contamination of the air with nasopharnyx bacteria (243). Greene and Vesley (247) measured the amounts of bacterial contaminants expelled before and after masking. Masked subjects expelled an average of 19 contaminants per 5 cu. ft., and 63% of these were less than 4 microns in diameter. Unmasked subjects expelled more than 5000 contaminants per

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5 cu. ft. and 72% of these were less than 4 microns in diameter. On the contrary, Cole, et al. (248) found that masking or complete exclusion of the nasopharnyx was ineffective in eliminating or significantly reducing air-borne bacteria. Their experiment showed that bacteria shed from the body surfaces were a greater source of air-borne bacteria than those from the nose and throat.

Daily bathing with hexachlorophene led to a reduction in the pre-shower aerial organisms shed by a majority of subjects (249). But this practice did not give consistent results and, therefore, cannot be relied upon as a means of control. The increase in bacterial shedding caused by shower bathing may be eliminated and the number of bacteria ordinarily shed reduced by: (a) application of 70% ethyl alcohol or lanolin to the skin, (b) by clothing the subject in a one-piece suit of tightly woven fabric (245).

Several procedures were enumerated by Munkacsy (250) to overcome the direct causes of contamination in clean rooms. These were:

(a) excessive coughing or sneezing was not permitted;
(b) smoking or eating was prohibited in all work areas;
(c) personal articles normally carried in the pockets such as keys, watches, coins, handkerchiefs, tissues, cosmetics, etc. were not permitted;
(d) special dust-preventive clothing including boots, caps, and gloves were worn;
(e) special procedures must be observed in cleaning shoes and utilizing the air shower;

(f) fingernails were scrubbed and cosmetics removed;
(g) eyeglasses, if worn, were washed and dried with lint-free tissue prior to entering the clean room.

Mechanical barriers, such as surgical gloves were considered as the best method to control the shedding of bacteria by human beings (222).

PROCEDURE

The method used in the study consisted of two parts: I. Quantitative determinations, and II. Qualitative determinations. The quantitative determinations involved the sampling of air and the enumeration of the total air-borne microorganisms shed as well as the other selected organisms (staphylococci, coliforms and hemolytic-type organisms). The qualitative determinations consisted of the biochemical tests used for identifying the isolates from Part I.

I. Quantitative Determinations

A. The Air Sampling Chamber

The sampling chamber (see Figures 1a, 1b, 1c, and 1d) was devised by the author. It was made of plywood and measured 4 x 2 x 10 ft. The chamber had two compartments, one for accommodating the subject and the other for the actual sampling. Both compartments were equipped with high efficiency fiberglass filters (99.97% for 0.3 micron particles) and a fan at the top for flushing out the air.

The sampling compartment had a tapered end at the bottom which held the metal sampling probe connecting it to the air sampler. A sliding door divided the two compartments and in this door, there were two 6-inch circular

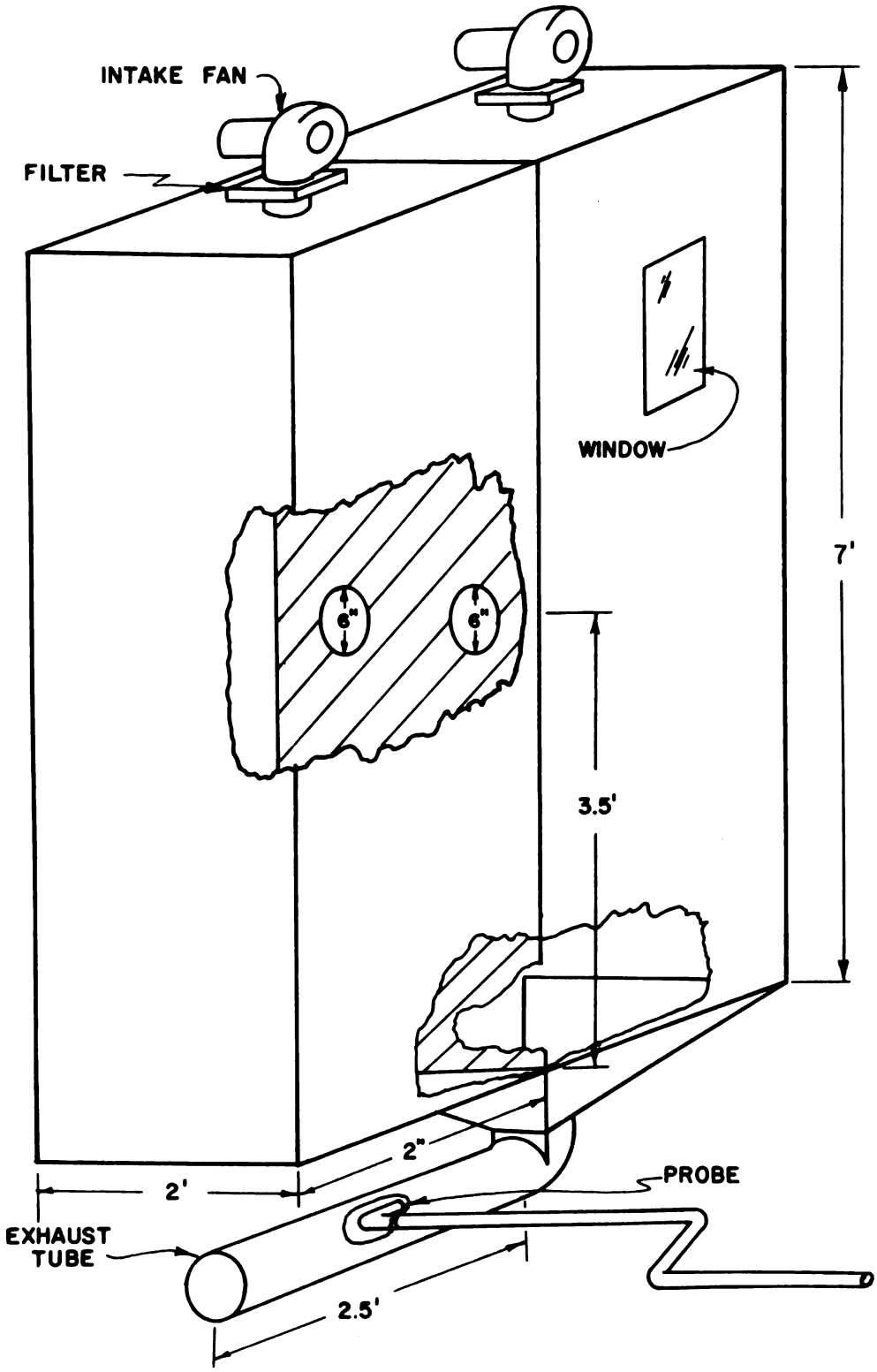

Fig. 1a. The sampling chamber.

Fig. 1b. The fans housing the high efficiency fiberglass filters.

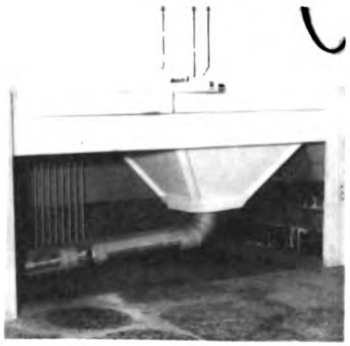

Fig. 1c. The tapered bottom of the sampling chamber with the metal sampling probe.

Fig. 1d. The interior of the sampling chamber.

Fig. 2. Muslin "sack sleeve", with one end open.

Fig. 3. Muslin sleeve with both ends open.

openings to allow the subject to expose his arm to air flow in the second compartment. These openings were designed to allow closure of one while the other was in use.

B. Disinfection of the Air Sampling Chamber

Approximately 20 ml. of propylene glycol were allowed to volatilize in both compartments overnight. Before each experiment, the chamber was flushed out with filtered air at a flow rate of more than 25 cu. ft. per min. for 20 to 30 min. Between collection of air samples, the chamber was flushed with filtered air for a few minutes.

As a control measure to check the sterility of the sampling compartment, 5 cu. ft. of air were sampled before each trial was run.

C. Preparation of a Subject

Four subjects were selected, one from each of the following operational areas of the dairy plant: milk, ice cream, cottage cheese and butter, and quality control laboratory.

A standard procedure was used to prepare both arms and hands of the subject for the experiment. One arm and hand, termed "Treated Arm" (TA) was given a preliminary wash, steps were as follows:

1. the hands and forearms were wet;
2. two ml. of Phisohex were rubbed on the hand and arm (to shoulder) for 1 min.;

3. small amounts of water were added to produce lather;
4. the nails were cleaned with a sterile brush and Phisohex[3] under running water; 25 strokes were used for each hand;
5. the entire arm and hand were then rinsed under running water.

The final wash steps were:

6. steps 2 and 3 were repeated but the arm and hand were scrubbed with a sterile gauze for 2 min., with small amounts of water added as needed;
7. the arm and hand were rinsed well under running water;
8. the entire arm and hand were sprayed with Zephiran Chloride[4] Solution (1:750);
9. then the arm and hand were dried with a sterile towel.

The TA was immediately placed in a specially-designed sterile muslin "sack sleeve" (See Figure 2) to protect it from contamination.

The other arm and hand, termed "Untreated Arm" (UTA) was washed by the following method:

1. the arm and hand were wet;
2. they were soaped until a good lather was obtained for 2 min., using a regular, white castille bar soap;
3. the arm and hand were rinsed under running water;
4. then they were dried with a disposable paper towel.

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[3]A synthetic, sudsing detergent and bacterial skin cleanser containing 3% hexachlorophene; brand name of Winthrop-Stearns Laboratories, Inc.

[4]Benzyl ammonium chloride type antiseptic and germicide, active against gram positive and gram negative organisms; brand name of Winthrop-Stearns Laboratories, Inc.

The subject proceeded to the sampling chamber immediately without touching anything.

D. Technique for Sampling the Air-borne Microorganisms

Air samples were collected at a rate of 1 cu. ft. per min. for 5 min. The flow rate of the air through the chamber was 5 cu. ft. per min., so only 20% of the air was sampled. The microorganism counts were expressed as numbers shed per minute[5]. This was the mean shedding rate per min. for a 5 min. sampling period.

An Andersen (six-stage) air sampler was used to collect the air enumerated for total air-borne microorganisms using Standard Plate Count Agar (Difco). The Casella Slit Sampler was used for collecting air samples on selective media to isolate staphylococci, coliforms and hemolytic-type organisms. The selective media[6] used were Mannitol Salt Agar, Violet Red Bile Agar and Blood Agar, respectively. Incubation was at 37°C. for 24 hr. for coliforms and 48 hr. for staphylococci and hemolytic-type organisms.

Rodac plates containing Standard Plate Count Agar without a neutralizer were used to determine the surface skin counts at the corresponding times when air samples were collected. A 30-second imprint was made on the middle of the

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[5]Obtained as follows:

$$\frac{\text{Number of air-borne microorganisms}}{5 \text{ cu. ft.}} \times \frac{5 \text{ cu. ft.}}{\text{min.}} = \text{no. of microorganisms shed per min.}$$

[6]All from Difco Laboratories.

outer arm adjacent to the elbow on a spot marked with a ball point pen.

E. Sampling Schedule

When the subject reached the chamber, a few minutes after the preparation of his arm, the Rodac sample was taken. The TA was exposed to the filtered air stream which was sampled for 5 min. using the Standard Plate Count Agar. This was followed by 5-min. samplings using Mannitol Salt Agar, Violet Red Bile Agar and Blood Agar, successively. This sequence was repeated using the UTA. The TA and UTA were again sampled alternately at approximately 30 min. and 60 min. from the initial exposure.

The sampling chamber was always flushed out with filtered air for a few minutes after each exposure of an arm. Meanwhile, the TA was kept covered with a sterile "sack sleeve" when not exposed to the filtered air.

After the morning sampling, a sterile disposable glove[7] was put on the TA while a sterile muslin sleeve (See Figure 3) covered the entire arm from the wrist to the shoulder. An ordinary pair of rubber gloves were then put on over the TA and the UTA, for additional protection against contamination and wetting. At the afternoon sampling, this entire outfit of the TA was removed in place of a ster-

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[7]Dispose-a-glove, Arbrook Division of Ethicon, Inc., Somerville, New Jersey.

ile sack sleeve. The UTA was left uncovered after removal of the rubber glove. A sampling schedule similar to the morning period was repeated. Sampling was performed once a week for each subject for a period of 5 wk. Temperature and relative humidity readings were recorded during the trials and are tabulated in Appendix 1. An average temperature of 20.4°C. and 22.4°C. for the morning and afternoon trials, respectively, were recorded. The average relative humidity readings for the morning trials were 56.3% and 52.0% for the afternoon trials.

During the 2 to 3 hr. interval between the morning and afternoon sampling, the subject was assigned to a single job in his respective working area in the dairy plant. This was done to control the subject's environment during the experiment.

II. Qualitative Determinations

A. Selection of Isolates for Identification Tests

Microbial colonies on the total air-borne microorganism plates that appeared different on visual observation by the author were isolated and held at 40°F. for further identification. The exceedingly large number of colonies collected made it impossible to identify every single one of them. Therefore, identification tests were made only on the microorganisms which were most numerous on any one sampling day (Refer to Appendix 2).

B. Methods of Identification

Routine tests for the identification of bacteria as recommended by the "Manual of Microbiological Methods" (251) were performed. A sample of the result sheets used is shown in Appendix 3. Incubation was at 32° to 35°C. The characteristics observed were:

1. Morphology
   a. Gram reaction
   b. Motility
2. Cultural Characters
   a. Nutrient Broth Culture
   b. Agar Colonies
3. Biochemical Tests
   a. Carbohydrate Fermentation
   b. Methyl Red Test
   c. Voges-Proskauer Test
   d. Hydrolysis of Starch
   e. Reduction of Nitrates
   f. Liquefaction of Gelatin[8]
   g. Production of Indole
   h. Production of Hydrogen Sulfide

[8]The incubated plate was flooded with a saturated solution of ammonium sulfate; clearing in the region of growth was positive.

RESULTS AND DISCUSSION

I. Quantitative Aspect

Microorganism Shedding Rates

The range of microorganism shedding rates obtained from the arms and hands of four dairy plant workers is shown in Table 2. The data for the TA and UTA for 5 sampling days are also presented. There is a noticeable variability in shedding rates between subjects. For example, Subject B has a shedding rate range of 6 to 57 per min., while Subject D has a shedding rate range of 1 to 10 per min. for the TA on the first testing day. For each subject, the range of the shedding rates also varied on different days. For example, for Subject A, the range of the shedding rates for the TA varied from 5 to 93 per min., 1 to 19, and 2 to 40 on the first, second, and third testing days, respectively.

A range of shedding rates of 0 to 95 per min. for the UTA and 0 to 93 for the TA were obtained. The lower value for the range does not necessarily represent the shedding rate immediately after washing or treatment of the arm.

There was a general overall increase in the mean shedding rates at the end of the afternoon sampling for both the

TABLE 2-Microorganisms shed per minute from arms and hands of human subjects on different days

Subject	Day 1		2		3		4		5		Overall	
	TA	UTA	TA	UTA	TA	UTA	TA	UTA	TA	UTA	TA	UTA
A	5-93	5-60	1-19	0-11	2-40	1-25	2-9	0-5	1-15	2-30	0-93	0-60
B	6-57	5-95	0-10	0-7	2-24	2-11	0-11	1-14	1-22	3-16	0-57	0-95
C	3-11	2-27	2-29	1-14	1-7	3-13	1-26	7-37	-	-	1-29	1-37
D[(a)]	1-10	1-13	2-8	0-9	0-25	1-9	0-4	0-4	1-25	0-7	0-25	0-13

[(a)] The use of medicated soap by Subject D before and during the trials may account for her lower bacterial counts. Ordinary bar soap was used by the other subjects.

TA and UTA (Figure 4). This was true for Subjects A, C, and D with respect to the TA (Figures 5a, 5e, 5g) and for Subjects A and C for the UTA (Figures 5b and 5f). No increase in shedding rates was shown by Subject B for the TA and UTA (Figures 5c and 5d) and by Subject D for the UTA (Figure 5h).

An increase in the number of bacteria shed during the afternoon sampling may have been due to the increased sweating of the subjects. Increased sweating could have: (a) assisted in the transport of more nutrients to the skin surface for aiding bacterial growth; (b) provided more moisture for growth, and (c) aided in the release of the microorganisms residing in the deeper layers of the skin. Different skin conditions may also have contributed to the different shedding rates obtained. Evans, et al.(197) found that under conditions of sweating and excess hydration, the total bacterial flora increased markedly. The subjects in this study used rubber gloves between the morning and afternoon sampling. In his study, Price (213) showed that the bacterial count of the skin surface increased 8.6 fold over the original count where rubber gloves were worn for 2 hr. and 40 min.

The overall increase in the mean shedding rate for the TA was slightly higher than for the UTA, being from 3.5 to 13.7 and from 5.8 to 11.8 per min., respectively, as shown in Table 3. The initial number of microorganisms shed from the TA was slightly lower than that of the UTA. This may

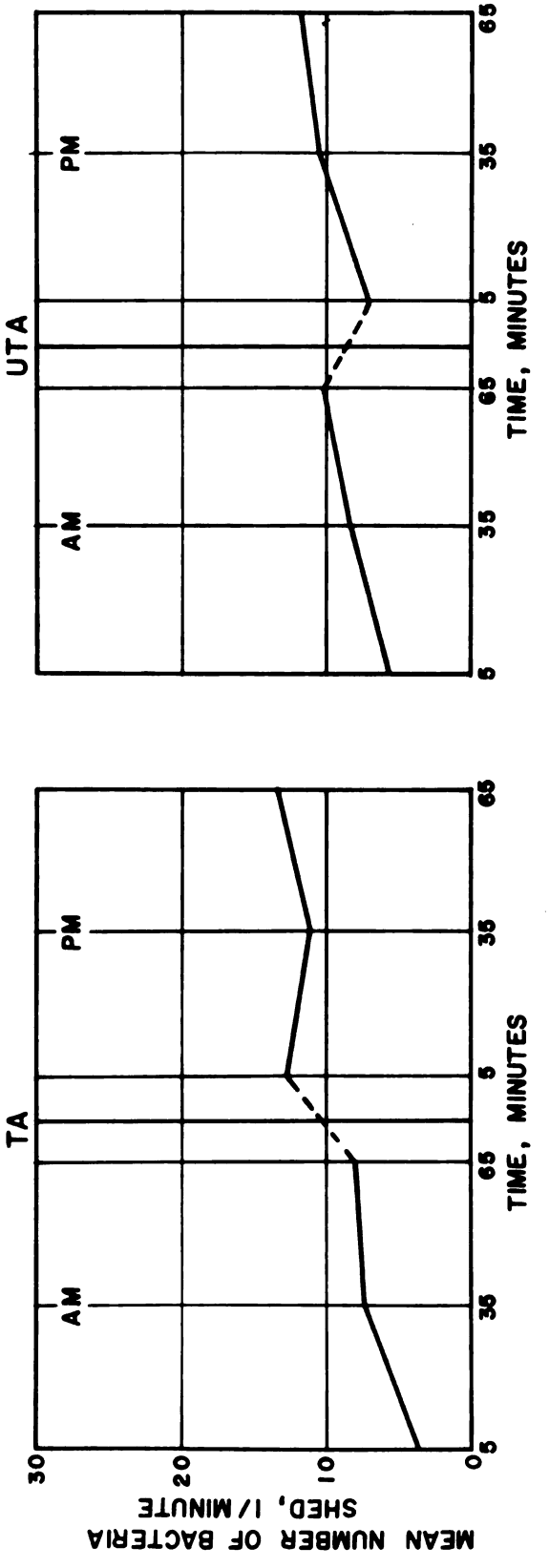

Fig. 4. Mean bacterial shedding rates for all subjects.

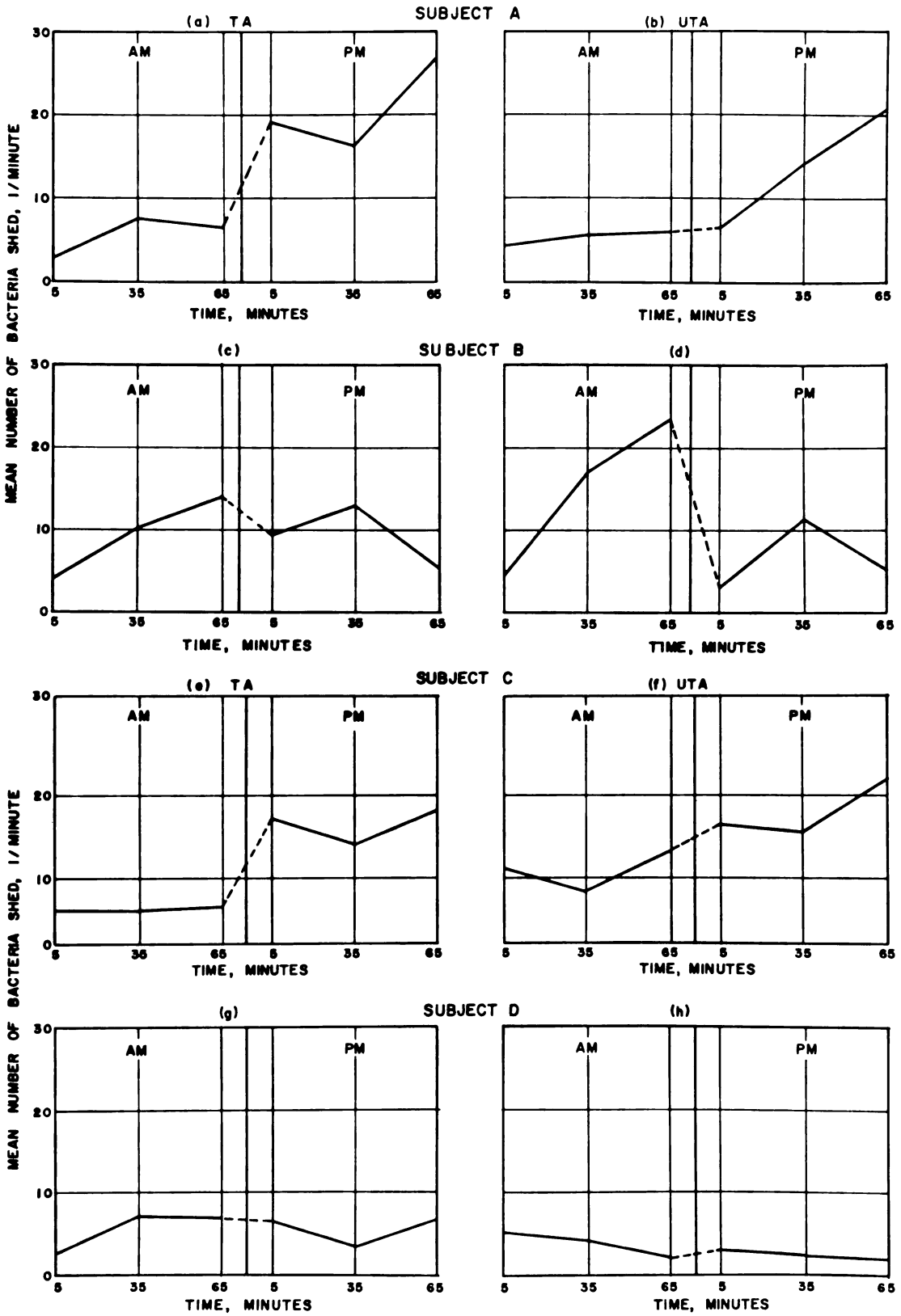

Fig. 5. Bacterial shedding rates for the different subjects.

TABLE 3-The average[(a)] number of microorganisms shed per minute by each subject at the various sampling periods

	Treated arm					
Minutes	AM 5	35	65	PM 5	35	65
Subject						
A	3.0	7.8	6.4	19.0	16.2	26.6
B	4.2	10.2	14.0	9.4	12.6	5.6
C	4.0	4.0	4.2	15.0	12.0	16.0
D	2.6	7.2	7.0	6.8	3.6	6.6
Total	13.8	29.2	31.6	50.2	44.4	54.8
Mean	3.5	7.3	7.9	12.6	11.1	13.7
	Untreated arm					
A	4.2	5.6	5.8	6.2	14.0	20.4
B	4.4	17.0	23.6	3.0	11.4	4.8
C	9.0	6.0	11.0	14.2	13.5	20.0
D	5.4	4.2	2.0	3.0	2.2	2.0
Total	23.0	32.8	42.4	26.4	41.1	47.2
Mean	5.8	8.2	10.1	6.6	10.2	11.8

[(a)] Obtained as the average number of microorganism counts from 5 sampling days.

be a reflection of the disinfecting efficiency of Phisohex over ordinary bar soap. Lowbury and Fox (252) found after testing several methods of hand disinfection that the use of Phisohex markedly reduced the surface counts, 2 min. after a 1-min. scrub.

The shedding rate ratio (S/S_o) was calculated from the microorganism shedding data. This ratio represents the rate at any time after the initial determination divided by the shedding rate immediately after washing or treating. From the least squares analysis of shedding rate ratios at different times, constants representing the slopes of the best least squares fit to the data have been calculated. These constants[9] for both the TA and UTA are shown in Table 4. They describe the rate at which the microorganism shedding changes with time. The results show that there are variations between the constants based on data from different subjects. The constants obtained for the TA are larger than those for the UTA. This would indicate that the shedding rate from the TA increased faster than from the UTA per min.

The number of microorganisms shed whether expressed as shedding rate or shedding rate ratio was found to be higher for the TA than the UTA. This is contrary to the author's expectation that the TA would have a lower count

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[9] S/S_o = Constant x Time x 1

TABLE 4-Constants[(a)] describing the influence of time on micro-organism shedding rate

Subject	Untreated arm (1/minute)	Treated arm (1/minute)
A	0.0164	0.0214
B	0.0037	0.0039
C	0.0113	0.0256
D	- 0.0018	0.0065

[(a)] Obtained from the least squares analysis of shedding rate ratios at different times.

due to the residual bacteriostatic effect of Phisohex used with the TA but not with the UTA. While an exact explanation for this observation is not known, perhaps the difference in moisture content between the two arms can partly account for this. The TA was kept covered with a sterile muslin sleeve at all times except during sampling periods. During the 2- to 4-hr. interval between the morning and afternoon sampling periods, a sterile plastic glove was worn under the rubber gloves together with a sterile muslin sleeve. On the other hand, the UTA wore only rubber gloves. This means that there may have been less air circulation with the TA resulting in accumulation of moisture on the skin from the subject's perspiration, which may have provided a more conducive environmental condition for growth of the microorganisms.

Viable Particle Size Distribution

The size distribution of the viable particles is one of the important characteristics of air-borne microorganisms. The size of the particle influences the settling rate of the particle. Figure 6 shows the size distribution of the viable particles shed by all the subjects. The largest percentage of the particles was in the range of 3.3 to 5.5 microns, for both the TA (26.43%) and UTA (28.65%). The same pattern applies for all subjects except for Subject D, as shown in Figure 7. The majority of the viable particles shed by the TA of Subject D were less than 1.0 micron

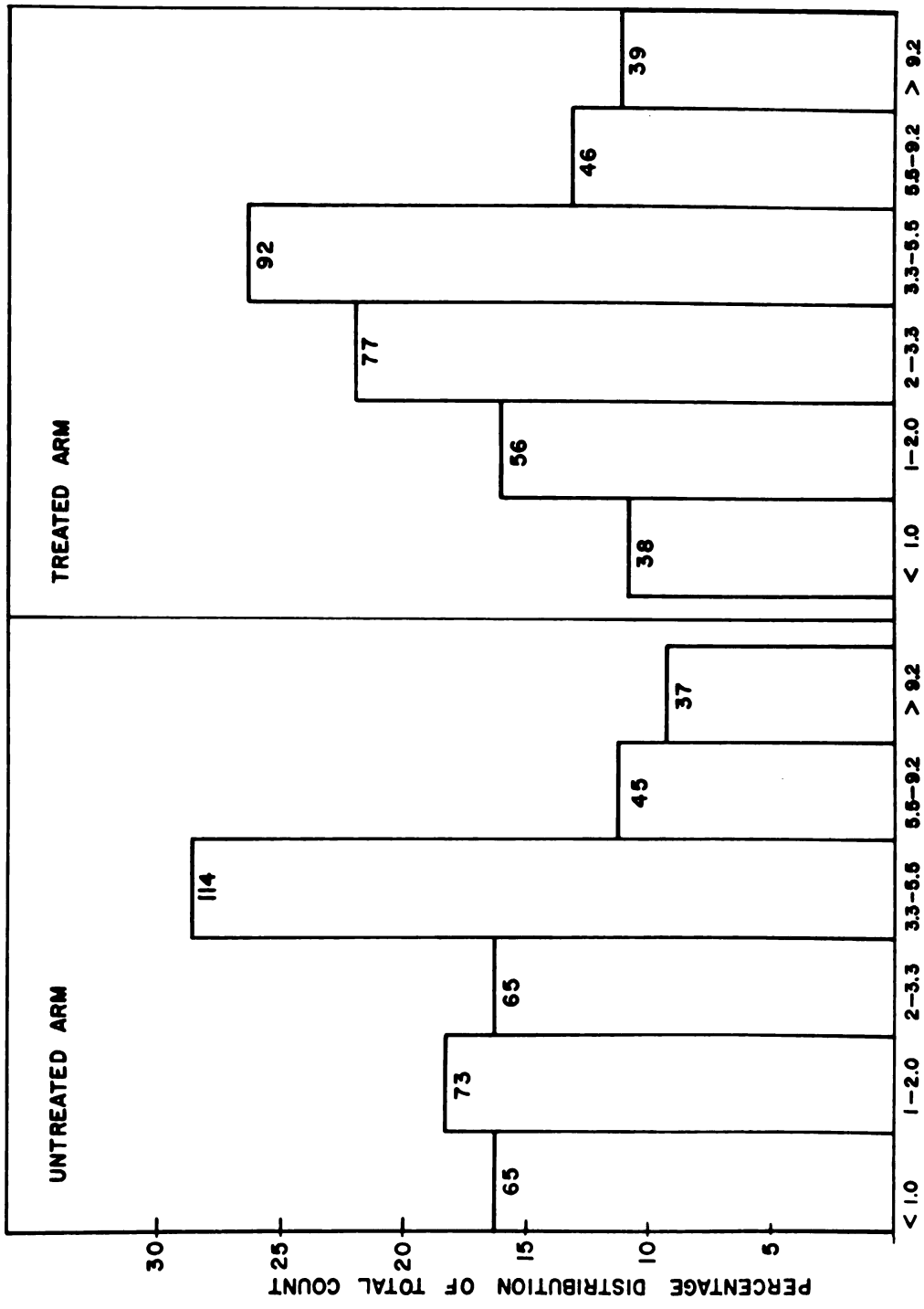


Fig. 6. Viable particle size distributions from untreated and treated arms and hands.

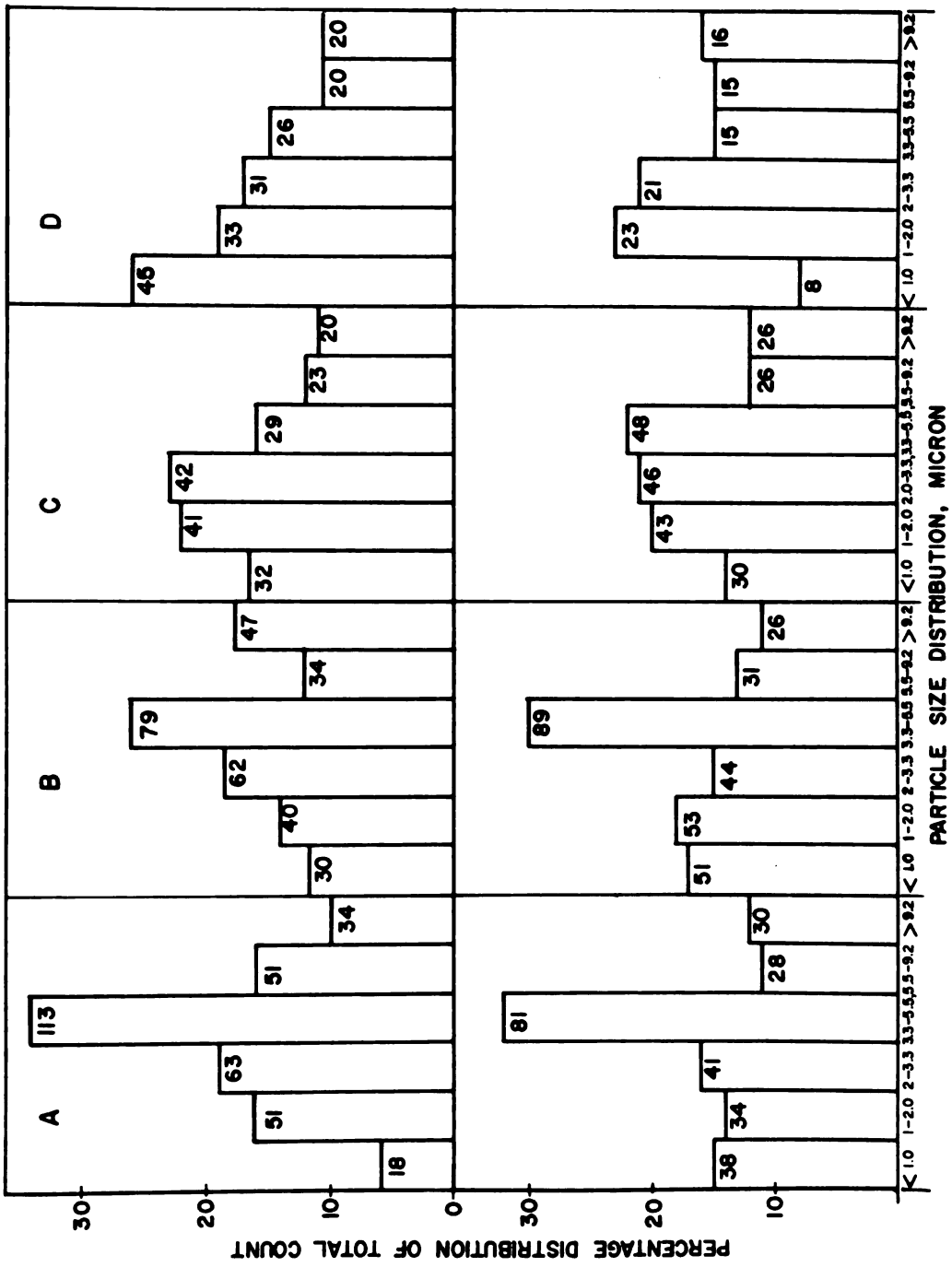


Fig. 7. Viable particle size distribution in air samples collected from untreated (lower graph) and treated (upper graph) arms and hands of four subjects.

in size. For the UTA, the greater part of the viable particles shed were in the size range of 1 to 3.3 microns. A possible explanation is that since Subject D produced less sweat, there was less moisture for the viable particles to imbibe. Therefore, smaller particles were shed since imbibition would tend to increase the size of the particles. Subjects A and B (Figure 7) shed a larger number of particles in the size range of 3.3 to 5.5 microns from the TA and UTA. Subjects C and D (Figure 7) shed particles of a more uniform size for both TA and UTA. The size distribution of the viable particles shed by each subject at different sampling periods for both the TA and UTA is shown in Appendices 5, 6, 7, and 8.

Influence of Skin Surface Population on Shedding Rate

To determine the influence of skin surface populations on shedding rates, contact plate counts were obtained at times corresponding to the times of shedding rate measurements. The data presented in Figure 8 show the overall effect of skin surface populations on shedding rates for all subjects. There is considerable scattering of the data but a least squares analysis indicates that shedding rate increases directly with skin surface population for both TA and UTA. This suggests that the number of organisms present on the skin surface influences the number of organisms that become air-borne. Contact plate sampling combined with air sampling has proven effective in the recognition of per-

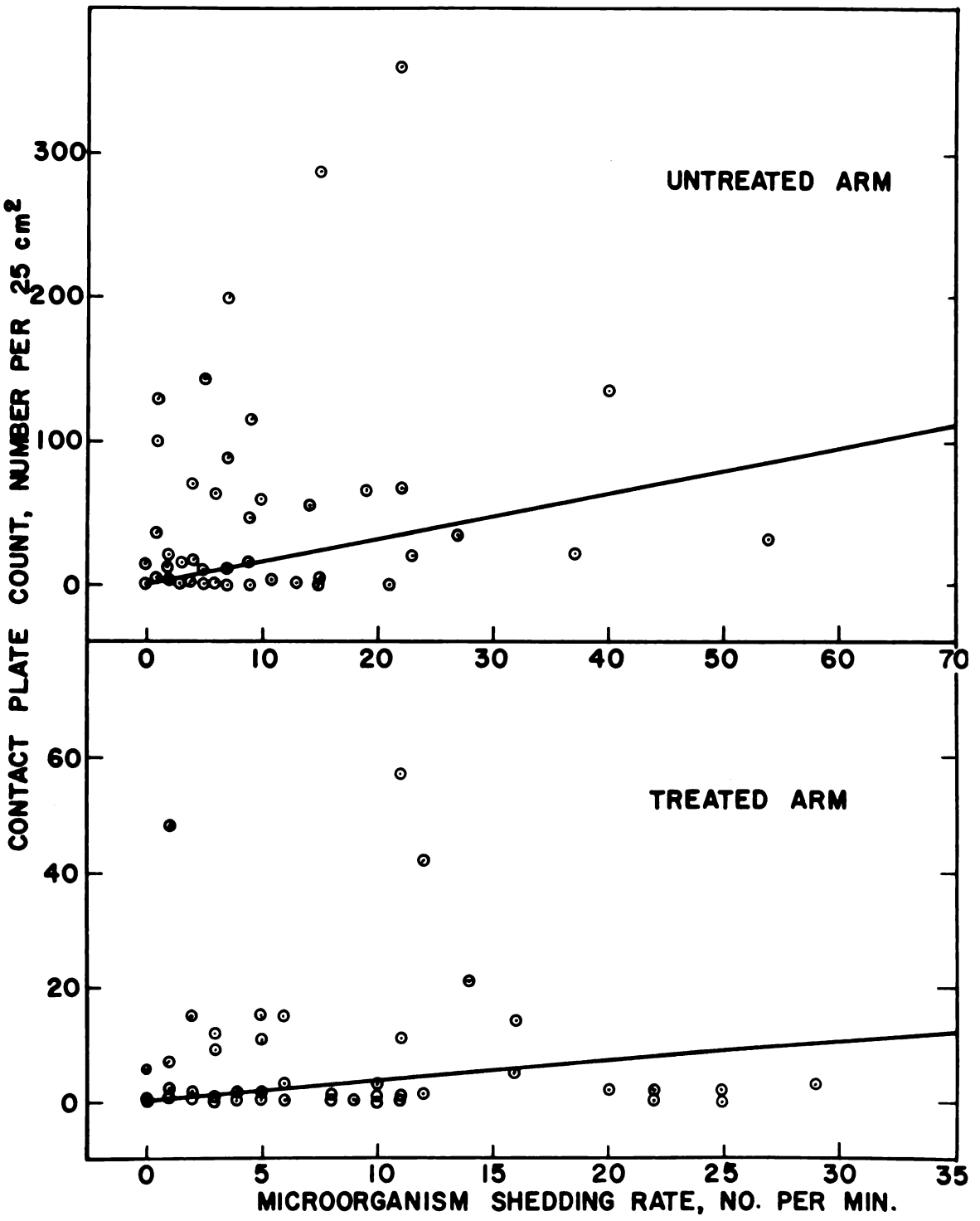


Fig. 8. Influence of arm surface microorganism count on shedding rates.

sonnel who are shedding potentially pathogenic organisms (221).

Regression coefficients of 1.573 for the UTA and 0.342 for the TA were obtained. The lower regression coefficient for the TA may have been due to the lack of a neutralizer in the Rodac plates used. Residual Phisohex and Zephiran Chloride may have been transferred onto the agar surface thereby inhibiting the growth of susceptible organisms.

Appendices 9 and 10 show the influence of time on surface plate counts for all subjects and for each individual subject for both the TA and UTA. Some low surface counts were obtained from the UTA, for example, Subjects B and D. No neutralizer was added to the agar in the Rodac plates which were used to obtain the surface counts of the TA. A limitation imposed by the use of contact plates is the prevention of any dispersal of clumps of bacteria, probably yielding a lower count.

II. Identification of Types of Microorganisms

Cocci and Rods

A summary of the organisms identified is shown in Table 5. Of the total 256 organisms identified, 55.4% were cocci, 41.4% were rods, and 3.2% were yeasts. A compilation of the occurrence of identified cocci from all subjects is presented in Table 6. The most common habitat of

TABLE 5-Summary of number of organisms identified

Subject	Rods	Cocci	Yeasts
A	35	63	5
B	8	20	1
C	40	23	0
D	23	36	2
Total = 256	106	142	8
% of total	41.4	55.4	3.2

TABLE 6-The occurrence of identified cocci

Cocci	No.	Common habitat[a]
Sarcina flava	40	air and water
Peptococcus prevotii	28	skin and human sources
Sarcina aurantiaca	27	air and water
Sarcina hansenii	16	water and dust
Staphylococcus epidermidis	11	skin
Sarcina ventriculi	5	dust
Sarcina lutea	4	air, water and skin
Micrococcus caseolyticus	3	dairy products like milk dairy utensils
Gaffkya tetragena	3	air and skin
Peptococcus saccharolyticus	2	skin of man
Staphylococcus aureus	1	skin and nasal mucous membrane
Micrococcus flavus	1	dairy products and utensils skin gland secretions
Micrococcus luteus	1	milk and dairy products dust

[a]According to Bergey's classification in the Manual of Determinative Bacteriology, (7th edition; Baltimore: The Williams and Wilkins Co., 1957).

the cocci are listed which were mainly the skin, air, water, milk and dairy utensils, as well as dust.

The occurrence of identified rods from all subjects is listed in Table 7. The common habitat of most of the rods are milk, skin, water, soil and dust. Three species originate from the intestinal canal.

It is interesting to note the frequent occurrence of microorganisms native to the dairy plant environment among the air-borne bacteria shed by dairy plant workers. This appears to be an illustration of the effect of environment on the skin flora of a person. Organisms normally alien to the skin but not to dairy products or utensils may become residents on the person's skin due to constant association and contact of the person's skin to the particular material or environment. In other words, persons exposed to high environmental contamination can become skin carriers and shedders. For example, nurses exposed in a ward in which Ps. aeruginosa was epidemic became skin carriers (252). Ulrich (221) observed the difference in bacterial populations between children and adults. Larger numbers of spore-forming bacilli were present on the skin of children. It was believed that the majority of spore formers were incidental contaminants picked from soil, rugs, or other similar materials.

Figures 9a and 9b and Tables 6 and 7 illustrate the frequency of occurrence, for each subject, of the cocci and

TABLE 7-The occurrence of identified rods

Rods	No.	Common habitat[a]
Alcaligenes marshallii	29	milk
Alcaligenes bookeri	22	intestinal canal
Pseudomonas synxantha	12	milk and cream
Pseudomonas iodinum	7	milk
Pseudomonas aeruginosa	6	polluted water and sewage
Corynebacterium striatum	4	mucous membrane
Alcaligenes recti	3	intestinal canal
Alcaligenes viscolactis	3	water, dairy utensils
Corynebacterium acnes	3	skin, hair follicles
Pseudomonas fluorescens	3	soil, water and food
Pseudomonas mildenbergii	3	air
Alcaligenes faecalis	2	decomposing matter
Alcaligenes metalcaligenes	2	intestinal canal
Corynebacterium pyogenes	2	abscesses in man
Corynebacterium xerosis	2	skin
Pseudomonas fragi	1	soil and water
Bacillus firmus	1	soil and decomposing matter
Bacillus megaterium	1	soil, water and dust

[a]According to Bergey's classification in the Manual of Determinative Bacteriology, (7th edition; Baltimore: The Williams and Wilkins Co., 1957).

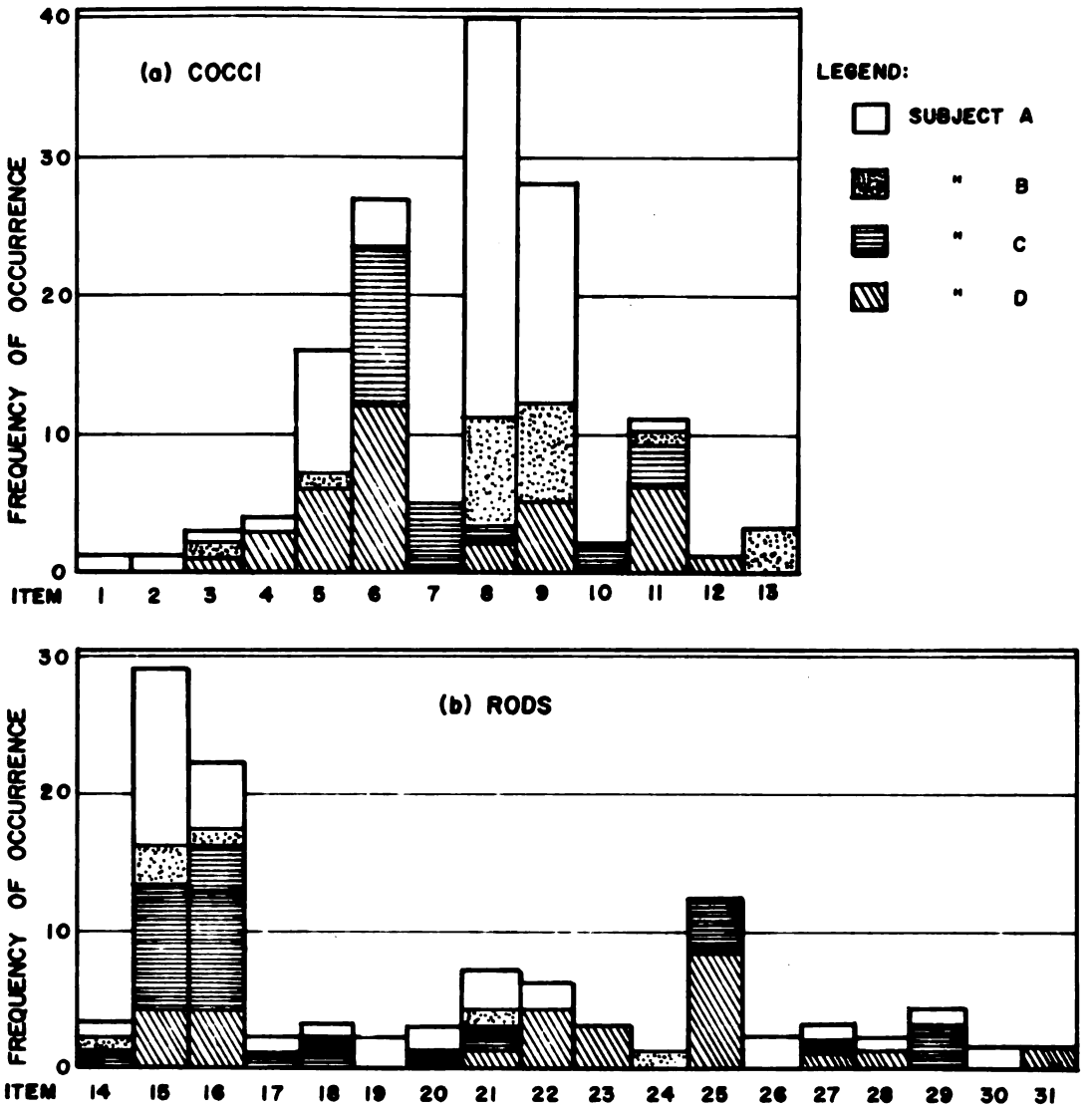


Fig. 9. Frequency of occurrence of the microorganisms identified according to subject.

1. M. flavus
2. M. luteus
3. M. caseolyticus
4. Sar. lutea
5. Sar. hansenii
6. Sar. aurantiaca
7. Sar. ventriculi
8. Sar. flava
9. Pep. prevotii
10. Pep. saccharolyticus
11. Staph. epidermidis
12. Staph. aureus
13. Gaff. tetragena
14. Al. recti
15. Al. marshallii
16. Al. bookeri
17. Al. metalcaligenes
18. Al. viscolactis
19. Al. faecalis
20. Ps. mildenbergii
21. Ps. iodinum
22. Ps. aeruginosa
23. Ps. fluorescens
24. Ps. fragi
25. Ps. synxantha
26. C. pyogenes
27. C. acnes
28. C. xerosis
29. C. striatum
30. Bac. firmus
31. Bac. megaterium

rods that were identified. Subject A accounted for 44.3% of all the cocci isolated. Subject C accounted for 38% of the rods while 33% were from Subject A. The majority of the cocci identified were comprised of: Sar. flava (40/142), Peptococcus prevotii (28/142), Sar. aurantiaca (27/142), Sar. hansenii (16/142), and Staph. epidermidis (11/142). The rods that were identified were mainly composed of Al. marshallii (29/106), Al. bookeri (22/106), and Ps. synxantha (12/106).

The frequency of the identified microorganisms according to source and time of shedding is shown in Figures 10a and 10b, and tabulated in Tables 8 and 9. The same number of cocci from the TA (am) and UTA (am) was identified. This was also true for the cocci isolated from the UTA (pm) and TA (pm). For the rods, 28 and 26 were identified from the TA (am) and (pm), respectively. Only 17 rods were identified from the UTA afternoon sampling and 35 were identified from the morning sampling.

The largest number of cocci identified was in the particle size range of 1 to 2 microns which was 26.5% of the total cocci identified (Figure 11). This was followed by a particle size range of 3.3 to 5.5 microns which was 20.2% of all the cocci identified (Refer to Table 10). For the rods, 32.7% of those identified were in the particle size range of 3.3 to 5.5 microns (Figure 11 and Table 11). Rods are expected to have a larger apparent diameter than cocci due to their inherent structure. The majority of the particles

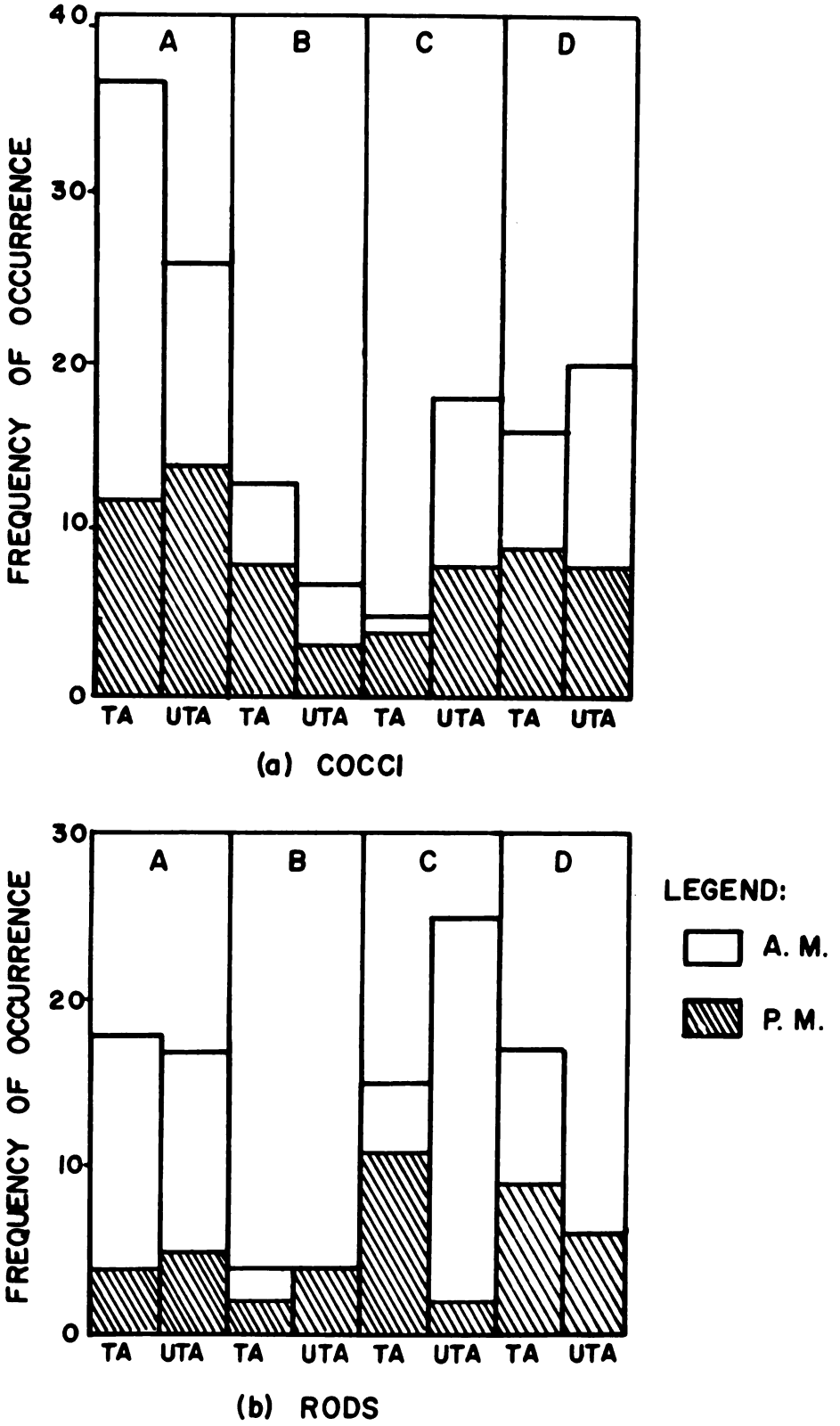


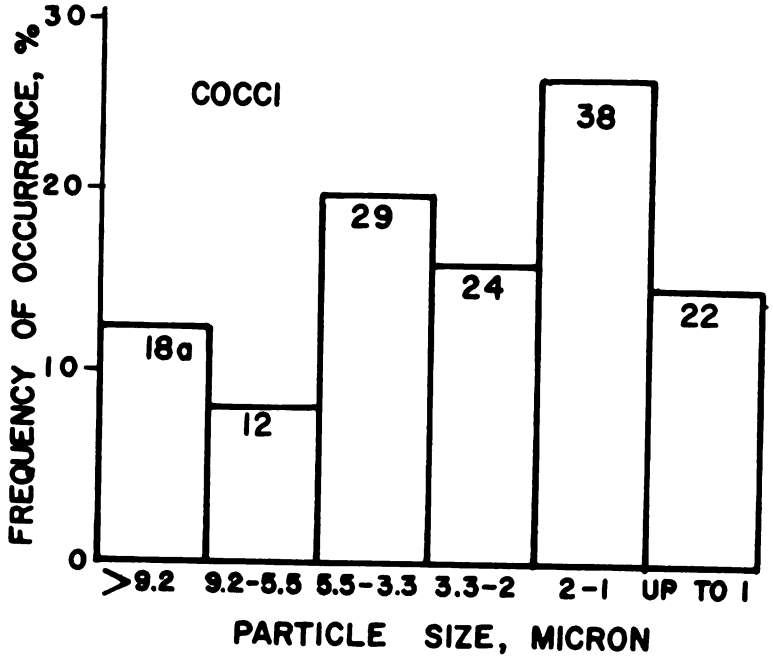
Fig. 10. Frequency of occurrence of the identified microorganisms according to the source and time of shedding.

TABLE 8-Summary of cocci identified from the treated arm and untreated arm

Source of organism	Treated arm		Untreated arm	
Period of sampling	AM	PM	AM	PM
M. flavus	-	-	-	1
M. luteus	-	1	-	-
M. caseolyticus	1	2	-	-
Sar. lutea	1	-	3	-
Sar. flava	15	7	9	9
Sar. hansenii	4	7	5	-
Sar. aurantiaca	6	5	12	4
Sar. ventriculi	-	2	2	1
Pep. prevotii	6	6	9	13
Pep. saccharolyticus	-	-	1	1
Staph. epidermidis	5	1	1	4
Staph. aureus	-	-	1	-
Gaffkya tetragena	-	2	1	-
Sub-total	38	33	38	33
Total	71		71	

TABLE 9-Summary of rods identified from the treated arm and untreated arm

Source of organism	Treated arm		Untreated arm	
Period of sampling	AM	PM	AM	PM
Al. bookeri	2	4	9	7
Al. faecalis	-	-	1	1
Al. marshallii	10	7	9	3
Al. metalcaligenes	-	-	2	-
Al. recti	2	1	-	-
Al. viscolactis	-	2	1	-
Bac. firmus	-	-	1	-
Bac. megaterium	-	1	-	-
C. acnes	-	3	-	-
C. pyogenes	1	-	1	-
C. striatum	-	1	3	-
C. xerosis	-	1	-	1
Ps. aeruginosa	3	-	3	-
Ps. fluorescens	2	1	-	-
Ps. fragi	-	-	-	1
Ps. iodinum	2	2	2	1
Ps. mildenbergii	1	-	1	1
Ps. synxantha	5	3	2	2
Sub-total	28	26	35	17
Total	54		52	



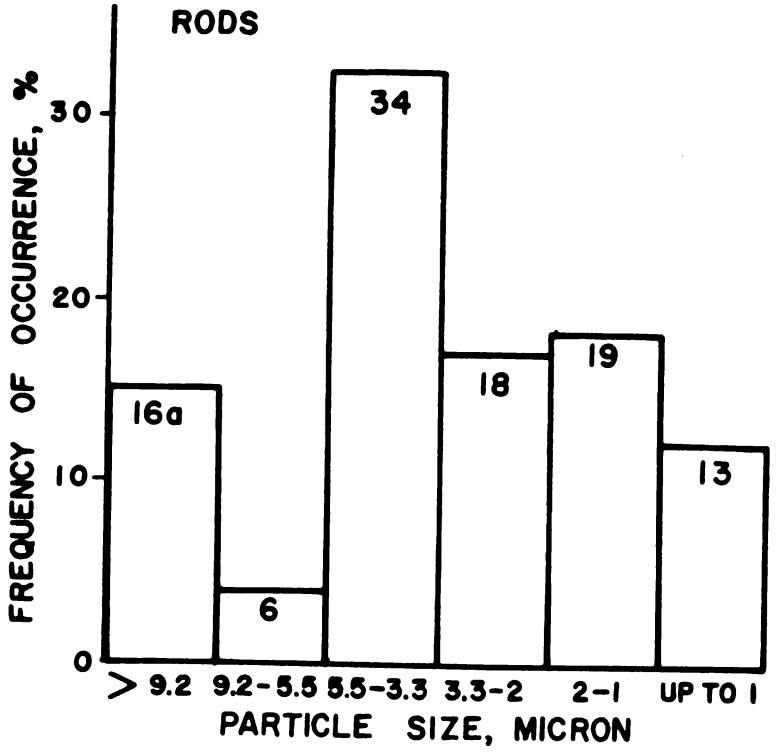


Fig. 11. Frequency of occurrence and size of the microorganisms identified.

TABLE 10-Frequency of occurrence of cocci in the various particle size ranges of the Andersen sampler

Andersen stage	1				2				3	
Particle size (μ)	>9.2				9.2-5.5				5.5-	
Subject	A	B	C	D	A	B	C	D	A	B
M. flavus										
M. luteus										
M. caseolyticus	1									
Sar. lutea				1				1		
Sar. hansenii	1	1							3	
Sar. aurantiaca	1			2			1		1	
Sar. ventriculi			1							
Sar. flava	4	1		1		2	1		4	
Pep. prevotii	1				3	2			5	
Pep. saccharolyticus										
Staph. epidermidis	1	1					1			
Staph. aureus										
Gaff. tetragena		1				1				
Sub-total	9	4	1	4	3	5	3	1	13	0
% of occurrence	12.6				8.3				20.2	
Total	18				12				29	

TABLE 10-Continued

3		4				5				6				
3.3		3.3-2				2-1				up to 1				
C	D	A	B	C	D	A	B	C	D	A	B	C	D	Total
						1								1
										1				1
1									1					3
		1											1	4
		1			3	2			3	2				16
1	3			7	2	1		2	4	1			1	27
2								2						5
		2				13	2			6	3		1	40
	4	4	1			2	1			1	3		1	28
				1								1		2
1	3			1					3					11
	1													1
							1							3
5	11	8	1	9	5	19	4	4	11	11	6	1	4	142
		16.6				26.5				15.3				
		23				38				22				142

TABLE 11-Frequency of occurrence of rods at the various particle size ranges of the Andersen sampler

Andersen stage	1				2				3	
Particle size (μ)	>9.2				9.2-5.5				5.5-	
Subject	A	B	C	D	A	B	C	D	A	B
Al. recti	1		1							
Al. marshallii	2	1							4	
Al. bookeri				1	1		1	3	2	
Al. metalcaligenes									1	
Al. viscolactis	1									
Al. faecalis	1									
Ps. mildenbergii										
Ps. iodinum		1		1						
Ps. aeruginosa	2		1							
Ps. fluorescens				1						
Ps. fragi						1				
Ps. synxantha				1						
C. pyogenes	1									
C. acnes									1	
C. xerosis									1	
C. striatum										
Bac. firmus									1	
Bac. megaterium										
Sub-total	8	2	2	4	1	1	1	3	10	0
% of occurrence	15.2				4.2				32.7	
Total	16				6				34	

TABLE 11-Continued

3		4				5				6				
3.3		3.3-2				2-1				up to 1				
C	D	A	B	C	D	A	B	C	D	A	B	C	D	Total
											1			3
5	2			3	1	5	2		1	2		1		29
3		1		6		1		1			1	1		22
1														2
1				1										3
						1								2
1						1				1				3
2		1				2								7
1				1								1		6
	2													3
														1
2	2			1				1	2				3	12
						1								2
												1	1	3
									1					2
2			1	1										4
														1
					1									1
18	6	2	1	13	2	11	2	2	4	3	2	4	4	106
		17.1				18.1				12.3				
		18				19				13				106

shed by the subjects studied were in the particle size range of 3.3 to 5.5 microns. This agrees with the earlier observation.

The author made a study of the air-borne microorganism numbers in different areas in the dairy plant (171). The subjects used in this study worked in the same areas. Sixty-two per cent of the viable particles collected from the various food packaging areas studied were in the particle size ranges of 1 to 5.5 microns. The influence of environmental contamination on the skin microflora may be involved.

The corresponding occurrence of a particular species of organism to a particular size range is not consistent. This is shown in Tables 10 and 11. For example, Staph. epidermidis in Table 10 was isolated from five different particle size ranges. This may be due to the different environmental and skin conditions that the organism was subjected to before being shed. Thus, it would be difficult to group the species of microorganisms shed, on the basis of particle size.

Yeasts

Eight out of the 256 organisms (3.2%) identified were yeasts. The sources of the yeasts identified were:

	Treated arm		Untreated arm	
	am	pm	am	pm
Number of isolates	3	2	0	3

Subjects	A	B	C	D
Number of isolates	5	1	0	2

Subject A yielded the highest number of yeasts while Subject C did not yield any. More yeasts were obtained from the TA (5) than from the UTA (3). Six of the yeasts were probably saccharomyces and two were candida, according to visual and biochemical tests.

Other Selected Organisms

Probable staphylococci, hemolytic organisms and coliforms were enumerated on selective media. The results are presented in Tables 12 and 13. Subject A yielded the highest number (22) of probable staphylococci. Twice as many were isolated from the TA than the UTA. No definite explanation can be given on why the TA of Subject A shed more probable staphylococci than the UTA. Probably, Subject A was a nasal carrier of staphylococcus and the organisms may have become part of the resident flora. But why the UTA should not have yielded a higher count remains unanswered. More data from a larger number of subjects will be needed to establish a feasible explanation. Subject C yielded 8 probable staphylococci with equal numbers from both arms. Subjects B and D yielded 1 probable staphylococcus each from the TA and UTA, respectively.

Subject B shed the highest number of hemolytic organisms (70), Subject C shed 6, and Subjects A and D did not shed any. It was noted that on one particular sampling day, Subject B shed 18 hemolytic organisms from the UTA and 45 from the TA. The author observed that there was a slight

TABLE 12-A summary of the number of selected organisms shed from the treated and untreated arms for a period of five sampling days

Subject	A		B		C		D	
Source	TA	UTA	TA	UTA	TA	UTA	TA	UTA
Probable Staphylococci	15	7	1	0	4	4	0	1
Hemolytic organisms	0	0	46	24	3	3	0	0
Coliforms	0	0	0	0	0	0	0	0

TABLE 13-The frequency of occurrence of probable staphylococci, hemolytic organisms and coliforms shed

Organism	Probable staphylococci				Hemolytic organisms				Coliforms	
Subject	No. of samples		Mean of non-zero counts	Range of counts	No. of samples		Mean of non-zero counts	Range of counts	No. of samples	
	Total	with 0 counts			Total	with 0 counts			Total	with 0 counts
A	31	13	0.63	0-7	26	26	0	0	33	33
B	30	29	0.03	0-1	31	30	0.25	0-24	25	25
C	19	16	0.87	0-4	21	16	0.39	0-2	12	12
D	31	30	0.20	0-1	32	32	0	0	29	29

cut on the index finger of the TA of Subject B at the time of the trial.

No coliforms were isolated from the microorganisms shed by any of the four subjects. This was unexpected since these organisms are quite ubiquitous in nature. Perhaps they were absent in the air samples collected, or they did not grow on the Violet Red Bile Agar.

SUMMARY AND CONCLUSIONS

The number of microorganisms shed from the treated and untreated arms and hands of four dairy plant workers was determined in a specially-designed sampling chamber. The number of microorganisms shed per min. ranged from 0 to 95 organisms for the untreated arm (UTA) and 0 to 93 for the treated arm (TA). A general increase in the mean number of microorganisms shed during the afternoon sampling was observed for both TA and UTA. Lower initial counts were obtained from the TA than from the UTA.

The distribution of the viable particles based on six size ranges was as follows: 1.0 micron (10.91% from the TA and 16.28% from the UTA), 1 to 2 microns (16.09% from the TA and 18.37% from the UTA), 2 to 3.3 microns (22.10% from the TA and 16.28% from the UTA), 3.3 to 5.5 microns (26.43% from the TA and 28.65% from the UTA), 5.5 to 9.2 microns (13.21% from the TA and 11.27% from the UTA), 9.2 microns (11.20% from the TA and 9.10% from the UTA). Most of the viable particles shed by all subjects were between 3.3 to 5.5 microns.

A least squares analysis of the Rodac plate counts indicated that the shedding rate increased with skin surface population for both the TA and the UTA.

Two hundred and fifty-six of the organisms isolated were identified as follows: 55.4% cocci, 41.4% rods, and 3.2% yeasts. A majority of the cocci were Sarcina flava, Peptococcus prevotii, Sarcina aurantiaca, Sarcina hansenii, and Staphylococcus epidermidis, in decreasing order. The rods were composed mainly of Alcaligenes marshallii, Alcaligenes bookeri, Pseudomonas synxantha, in decreasing numbers. The microorganisms identified were native inhabitants of the skin, dairy products and utensils, water, soil, dust and intestinal canal.

The largest number (26.5%) of the cocci identified were in the particle size range of 1 to 2 microns, while 20.2% were in the particle size range of 3.3 to 5.5 microns. For the rods, 32.7% of those identified were in the size range of 3.3 to 5.5 microns and 18.1% were in the size range of 1 to 2 microns.

All four subjects yielded small numbers of probable staphylococci. Two subjects shed hemolytic-type organisms. No coliforms were isolated from the organisms that were shed.

RECOMMENDATIONS FOR FUTURE WORK

This Study has disclosed some valuable and interesting aspects of microorganism shedding by human subjects. But it has barely touched the surface. More research will be needed to establish facts and techniques, i.e., air sampling and bacteriological methods and the design of an ideal sampling chamber. The following are some of the recommendations for future work:

(a) Re-design a sampling chamber, probably of stainless steel with facilities for controlled environmental conditions, such as temperature, relative humidity, light, and ultraviolet light.

(b) Test the entire surface of the person instead of just the arm and hand.

(c) Use different flow rates of filtered air passing through the chamber and ascertain how this influences the total number of organisms isolated.

(d) Collect air samples for longer periods of time without dehydrating the culture media.

(e) Use a liquid impinger sampler and collect the air samples on an enrichment broth for resuscitation of the microorganisms and for purposes of incubation. After incubation, the broth is diluted out and plated on different se-

lective media as desired. This method may take longer than the solid impaction method but it may yield higher bacterial counts. The resuscitation period serves to revive damaged or weakened microorganisms.

(f) A study of the environmental aspect of microorganism shedding from the human skin is important. For example, a subject may be placed under different environmental conditions, i.e., pH, relative humidity, etc. to determine what the ideal environmental conditions are to induce the release of microorganisms from the skin to the environment.

(g) Design plant uniforms and laboratory coats for food plant workers that would eliminate microorganism shedding into the air, especially for use in aseptic areas in food processing plants. Determining the best material suited for this purpose would also be a part of this study.

(h) Another interesting study is how to isolate the species of the microorganism(s) present in an air-borne particle, if the size range is known. Of particular importance are the infectious organisms such as Staph. aureus.

(i) The influence of the person's environment on the microflora of his skin, particularly those that become air-borne is an important aspect to consider. More data are needed to re-affirm whether persons exposed to heavy contamination of certain microorganisms become skin carriers.

(j) More extensive data on the correlation between surface plate counts and the number of microorganisms shed by human subjects are needed. Furthermore, selective media may be used to isolate specific microorganisms. If this relationship could be established, then persons may be tested before being employed in a food plant to find out whether they are heavy emitters or not. For example, in the case of a heavy emitter, it would be unwise to place this person in an aseptic packaging line or in the final process of packaging pre-cooked perishable frozen foods such as custards and meat dishes with sauce.

(k) Establish satisfactory indices of microorganism contamination of the air of various food processing areas according to the operations performed therein. This would include a preliminary survey of the air-borne microorganism populations in different food processing plants.

APPENDIX

1. Temperature and relative humidity measurements recorded during the trials

Sampling date	Temperature (°C.)		Relative humidity (%)	
	AM	PM	AM	PM
4/8/66	-	26	-	44
9/8	22.5	23	50	45
10/8	22	22	48.5	49.5
11/8	19	-	57.5	-
12/8	20	22	56	45.5
16/8	21.5	22.5	70.5	62
17/8	21	23	63	60
18/8	22	25	65	65
19/8	21	22	53	50
23/8	18.5	19	58.5	55.5
24/8	19	-	56	-
25/8	19	19.5	57.5	55.5
26/8	19	22	60	55
30/8	22	24	56	55.5
31/8	22	24.5	56	53
2/9	23	24.5	59.5	54.5
6/9	18	20	50	45
8/9	18	20	46	45.5
9/9	19	22	50	43.5
Average	20.4	22.4	56.3	52.0
Range	18-23	19-26	46-70.5	43.5-65

2. The total number of microorganisms isolated from the airborne microorganism plate counts

Subject	Sampling day	Number of isolates from		
		TA	UTA	total
A	8.10.66	4	0	4
	8.17	4	7	11
	8.24	9	15	24
	9. 2	20	14	34
	* 9. 9	70	47	117
B	8. 9.66	21	9	30
	8.12	11	0	11
	8.19	7	8	15
	* 8.26	18	11	29
	8.31	5	4	9
C	8. 8.66	5	3	8
	8.16	7	8	15
	* 8.23	27	62	89
	8.30	19	5	24
	9. 5	18	18	36
D	8.11.66	6	10	16
	8.18	5	6	11
	* 8.25	44	32	76

*Being the most numerous, isolates from this particular sampling day were selected for further identification tests.

3. Result sheet used for tests on identification of microorganisms

ORGANISM_____ REFERENCE_____
SOURCE _____
MORPHOLOGY: Form_____ Arrangement_____ Size_____
Gram's stain_____ Capsules_____ Motility_____
Flagella_____ Spore formation_____
Colony on agar: Age_____ Density_____ Pigment_____
Size_____ Form_____ Margin_____ Elevation___
Surface_____ Consistency_____
Agar slopes: Age_____
Growth_____ Form_____ Elevation_____ Lustre_____
Surface____ Optical characters____ Chromogenesis_
Consistency_____ Odor_____
Nutrient broth: Age_____ Temp._____°C.
Surface growth_____ Amount of growth_____
Subsurface growth_____ Sediment_____

Biochemical Characteristics:

CHO fermentation: Temp._____°C. Medium_____

	Glu.	Lac.	Mal.	Suc.	Man.	Gal.	Raff.
acid___ days___							
gas____ days___							

Gelatin liquefication: Age_____ Temp._____°C. Medium_____
Rate_____ Type_____
Nitrate reduction: Age_____ Temp._____°C. Medium_____
Nitrate_____
Gas (N)_____
Indole production: Age_____ Temp._____°C. Medium_____
Method_____
Indole_____
Hydrogen sulfide production: Age_____ Temp._____°C.
H_2S_____ Medium_____
Starch hydrolysis: Age_____ Temp._____°C. Medium_____
Reaction_____
Methyl red test: Age_____ Temp._____°C. Medium_____
Reaction_____
Voges-Proak. test: Age_____ Temp._____°C. Medium_____
Reaction
Additional tests:

4. Summary of Federal Standard No. 209 - Industrial Clean Rooms

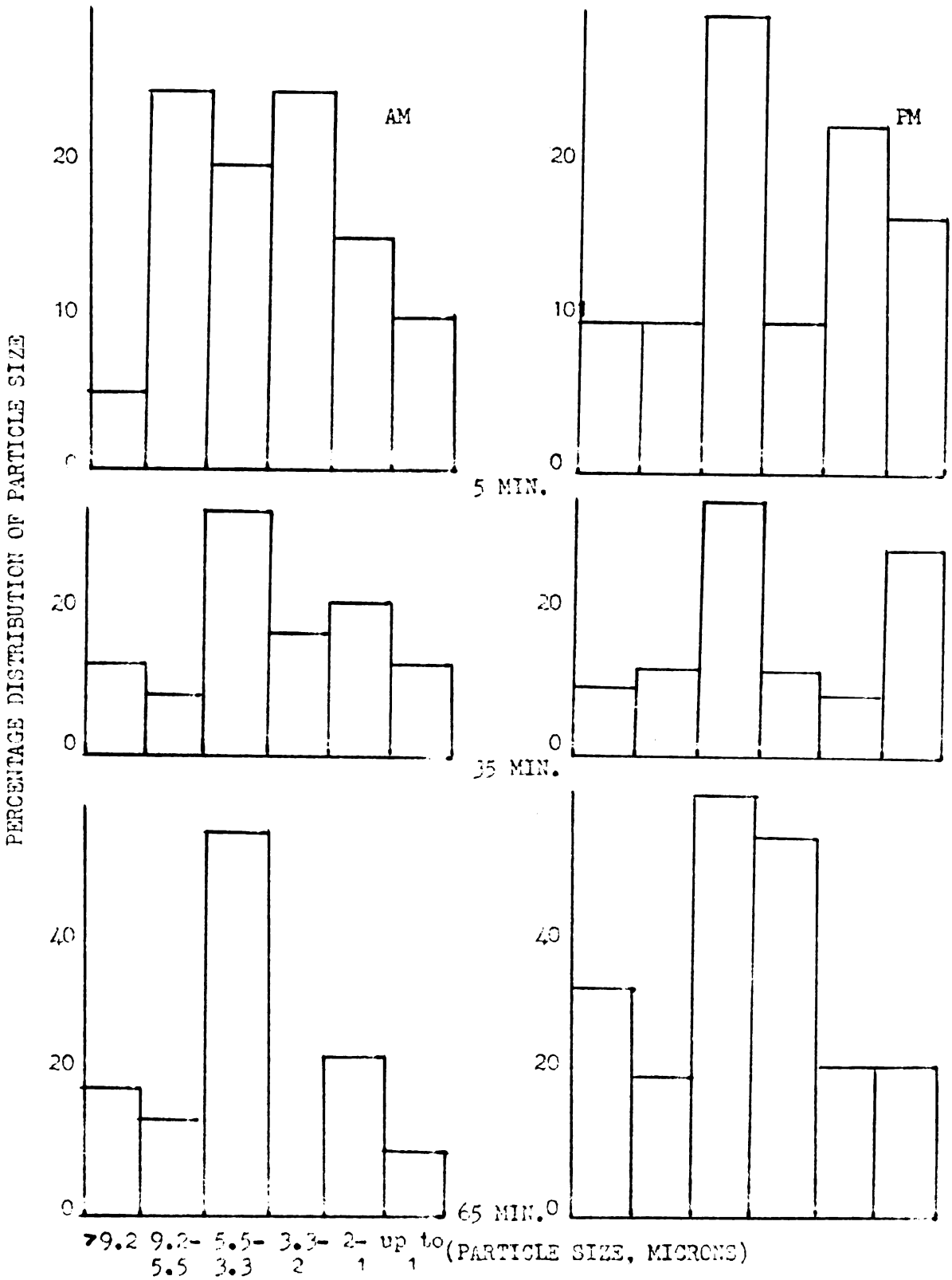
Maximum number of particles per cu. ft. (0.5 μ and larger)	Class	Maximum number of particles per cu. ft. (5.0 μ and larger)
100	100	-
10,000	10,000	65
100,000	100,000	700

Pressure: "all clean rooms shall maintain a pressure above that of surrounding areas to ensure that all leakage shall be outward".

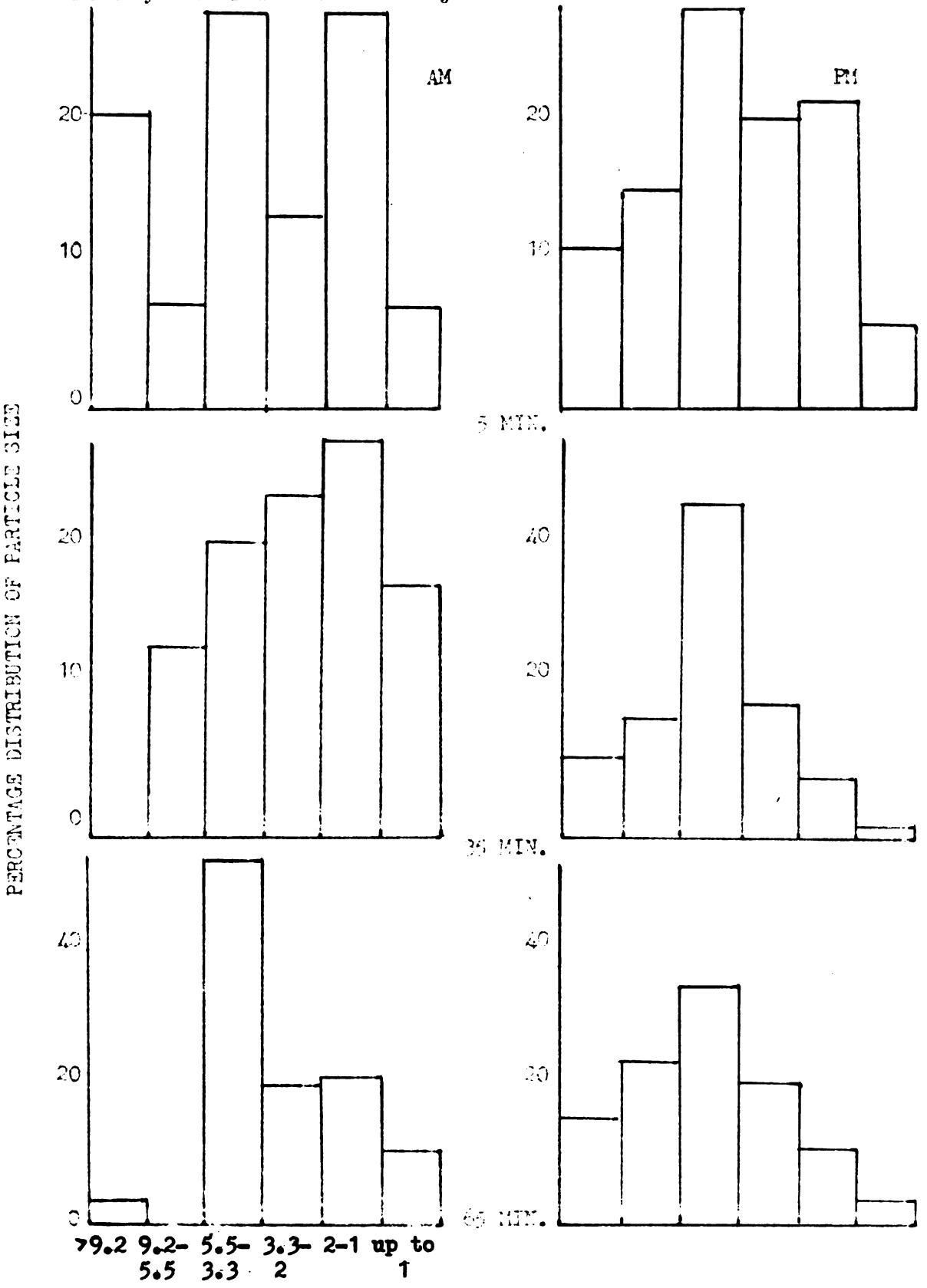
Temperature range: " temperature controls, if applicable, shall be capable of maintaining a specified temperature within a range of 67°to 77°F. within ±5°F. in less temperature critical areas, and up to ±0.5°F. in temperature sensitive applications ".

Humidity range: " the maximum relative humidity shall be 45%. Humidity controls shall be capable of holding a specified relative humidity within ±10% for general applications and up to ±5% for humidity sensitive applications ".

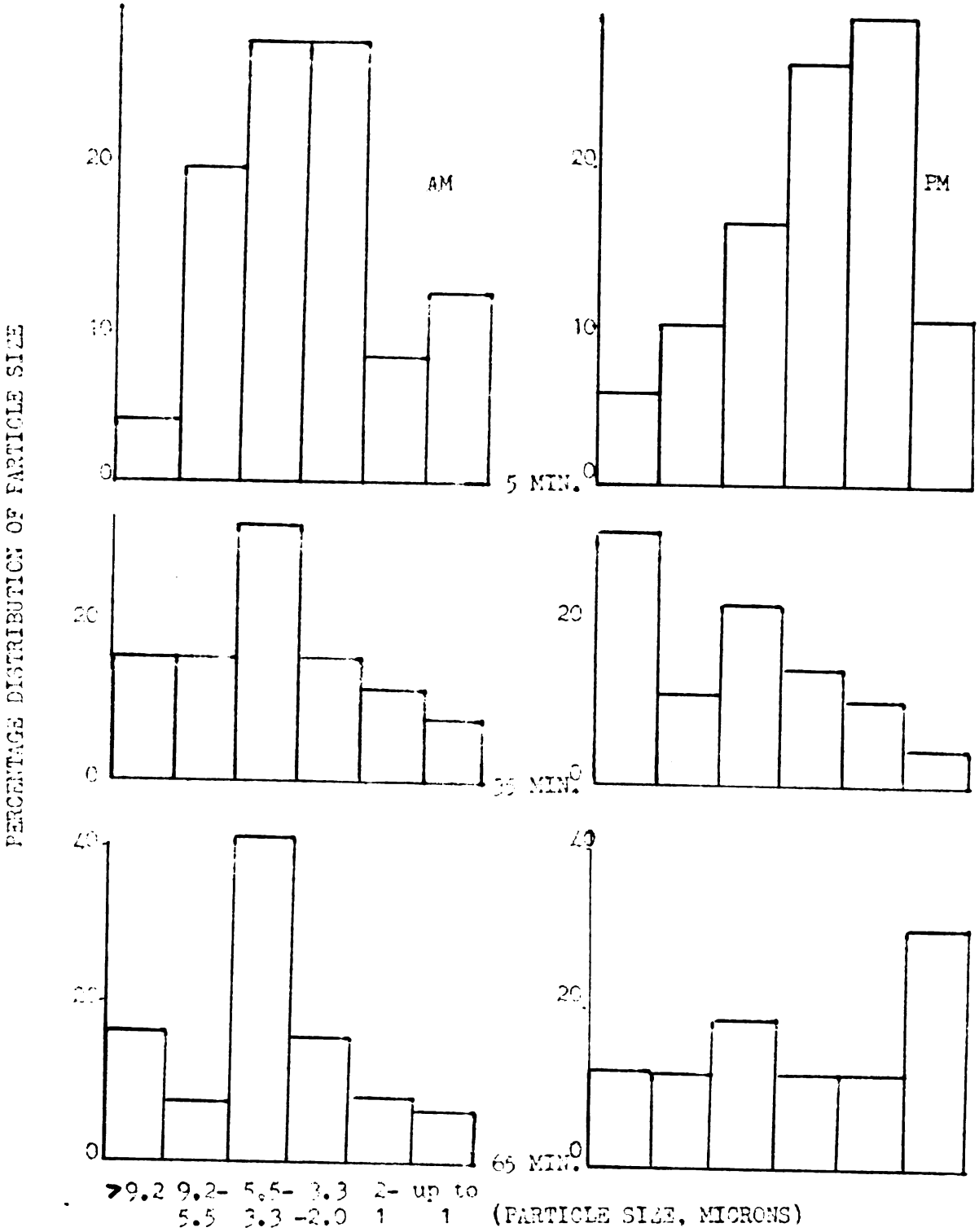
5. Particle size distribution of the air-borne microorganisms shed by the untreated arm of Subject A



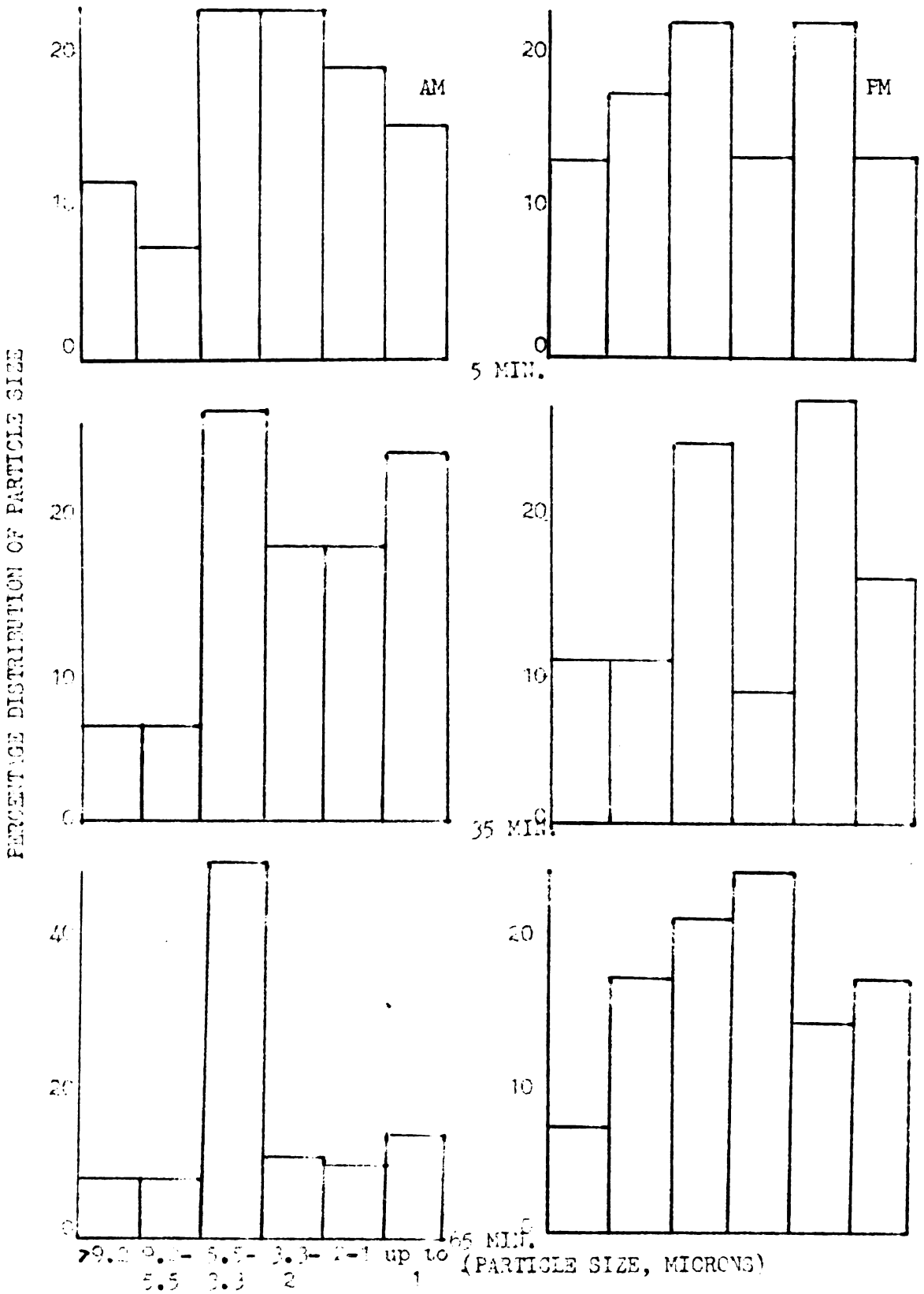
5. Particle size distribution of the air-borne microorganisms shed by the treated arm of Subject A



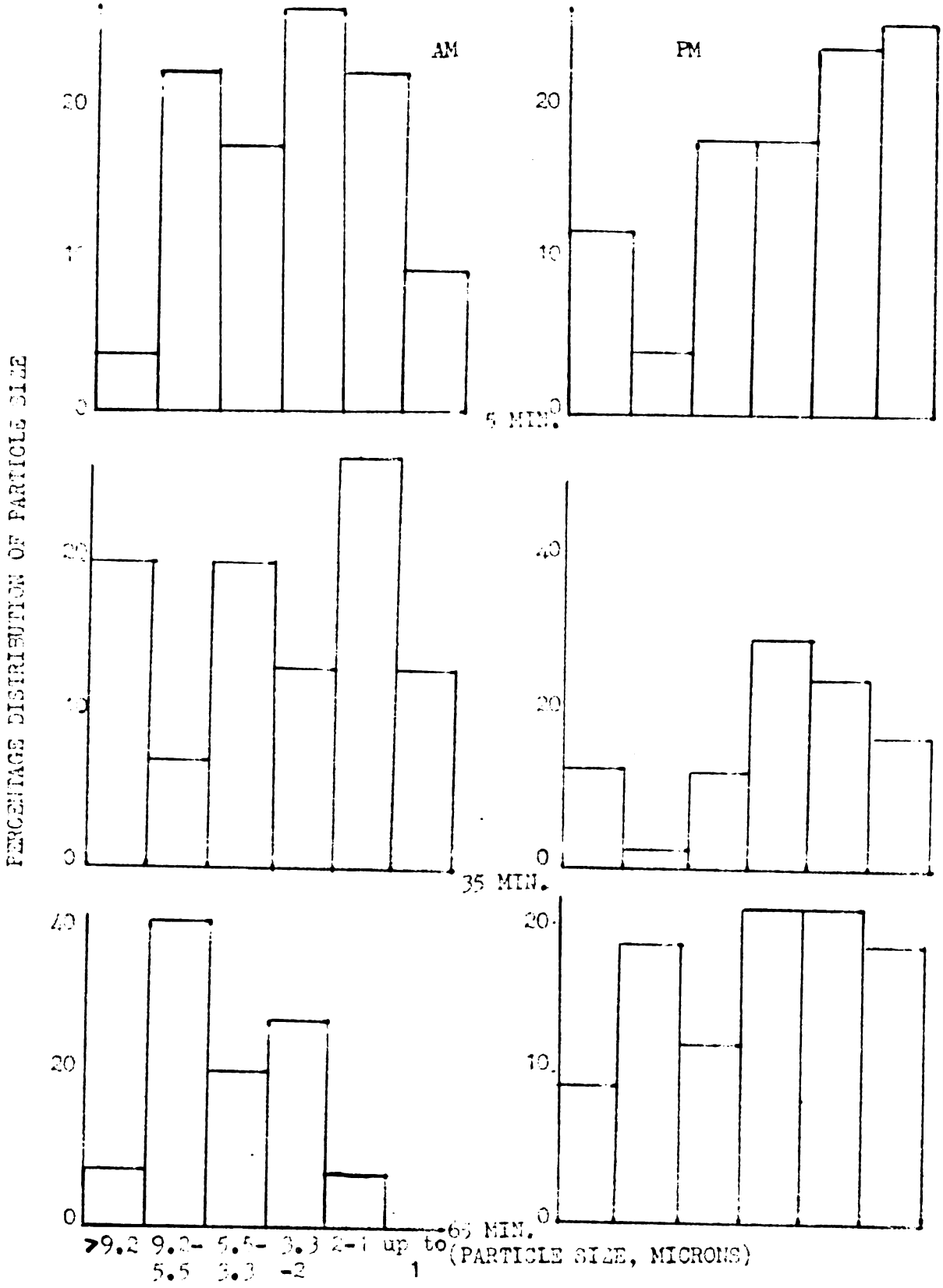
6. Particle size distribution of the air-borne microorganisms shed by the treated arm of Subject B



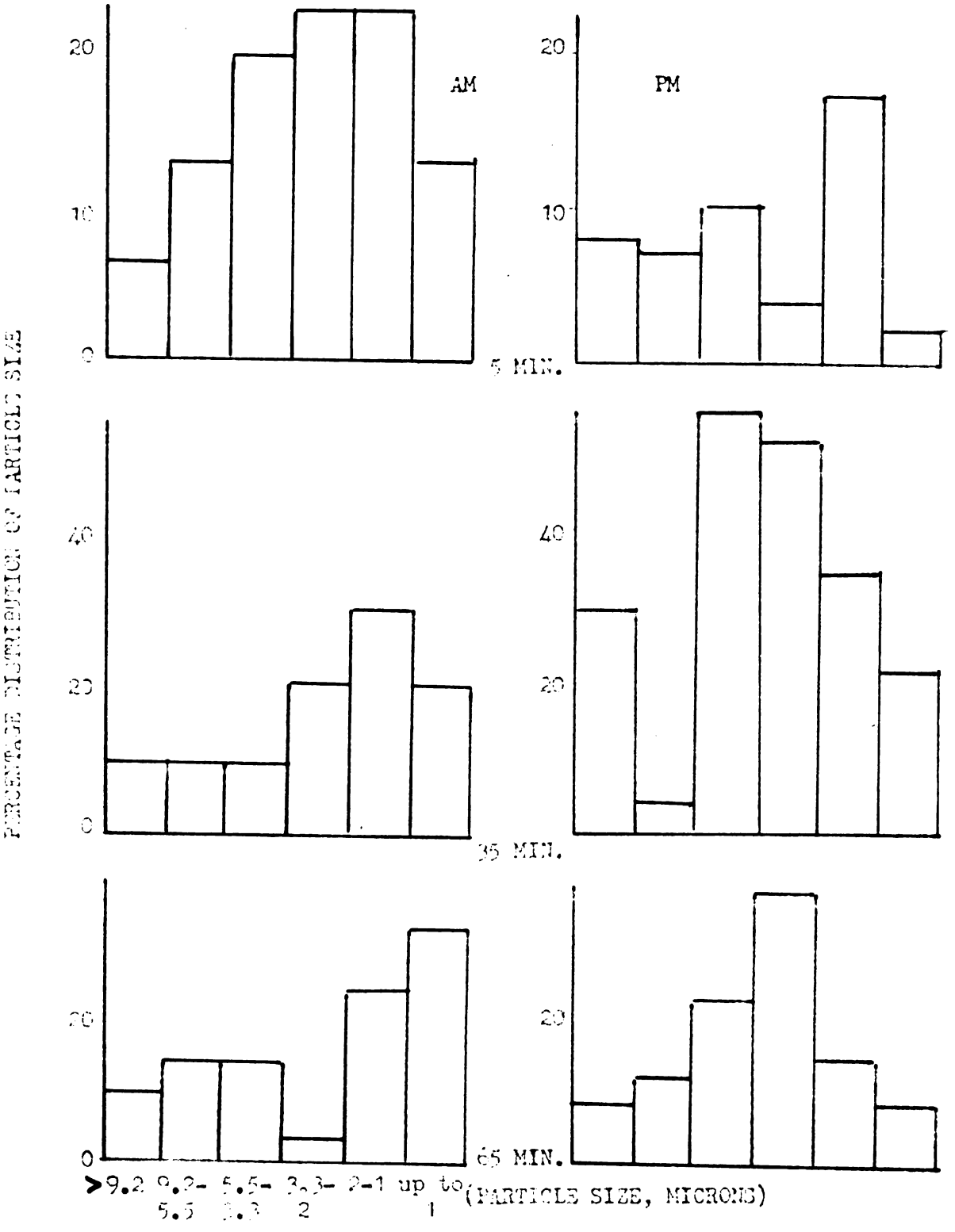
6. Particle size distribution of the air-borne microorganisms shed by the untreated arm of Subject B



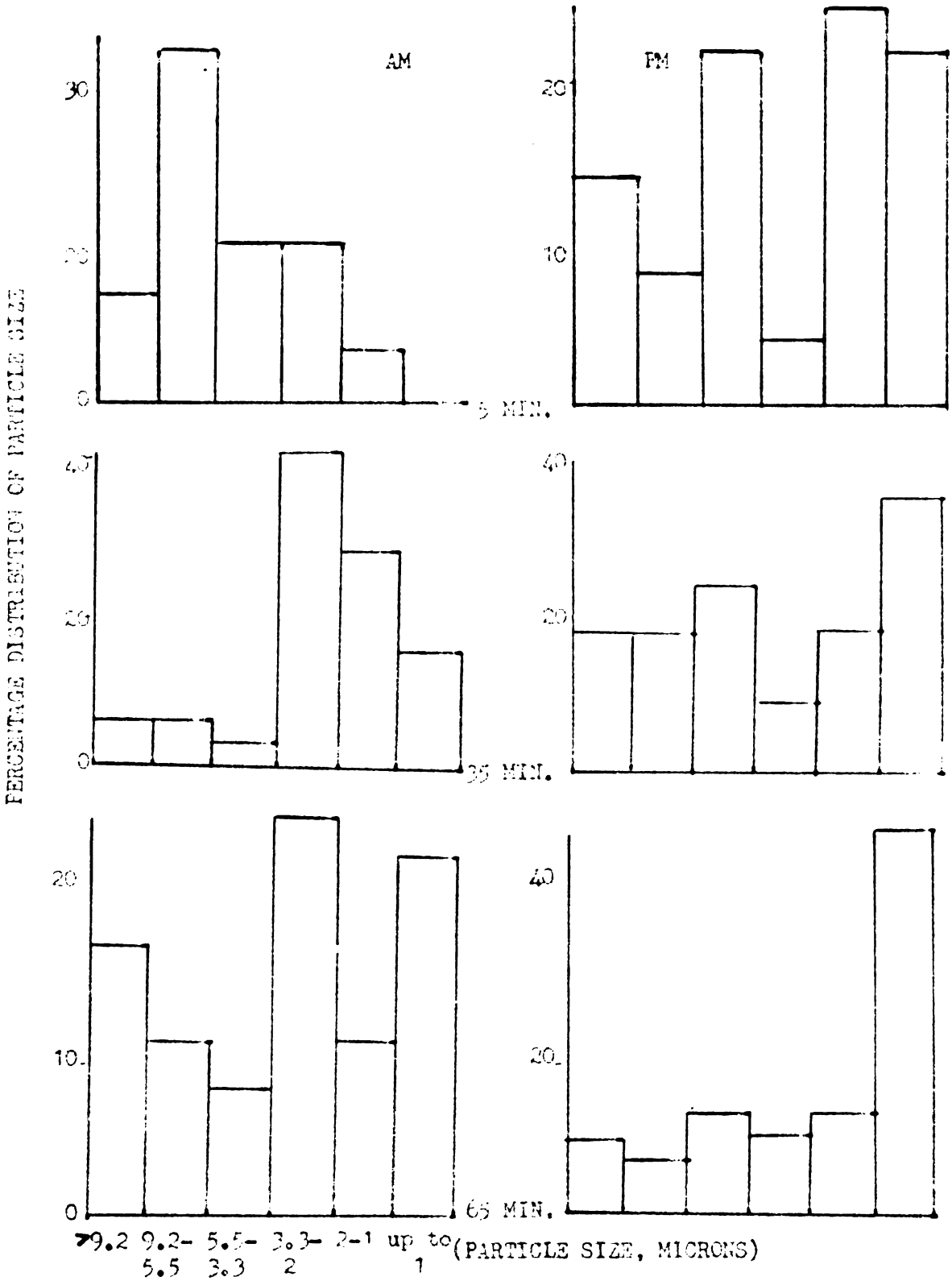
7. Particle size distribution of the air-borne microorganisms shed by the treated arm of Subject C



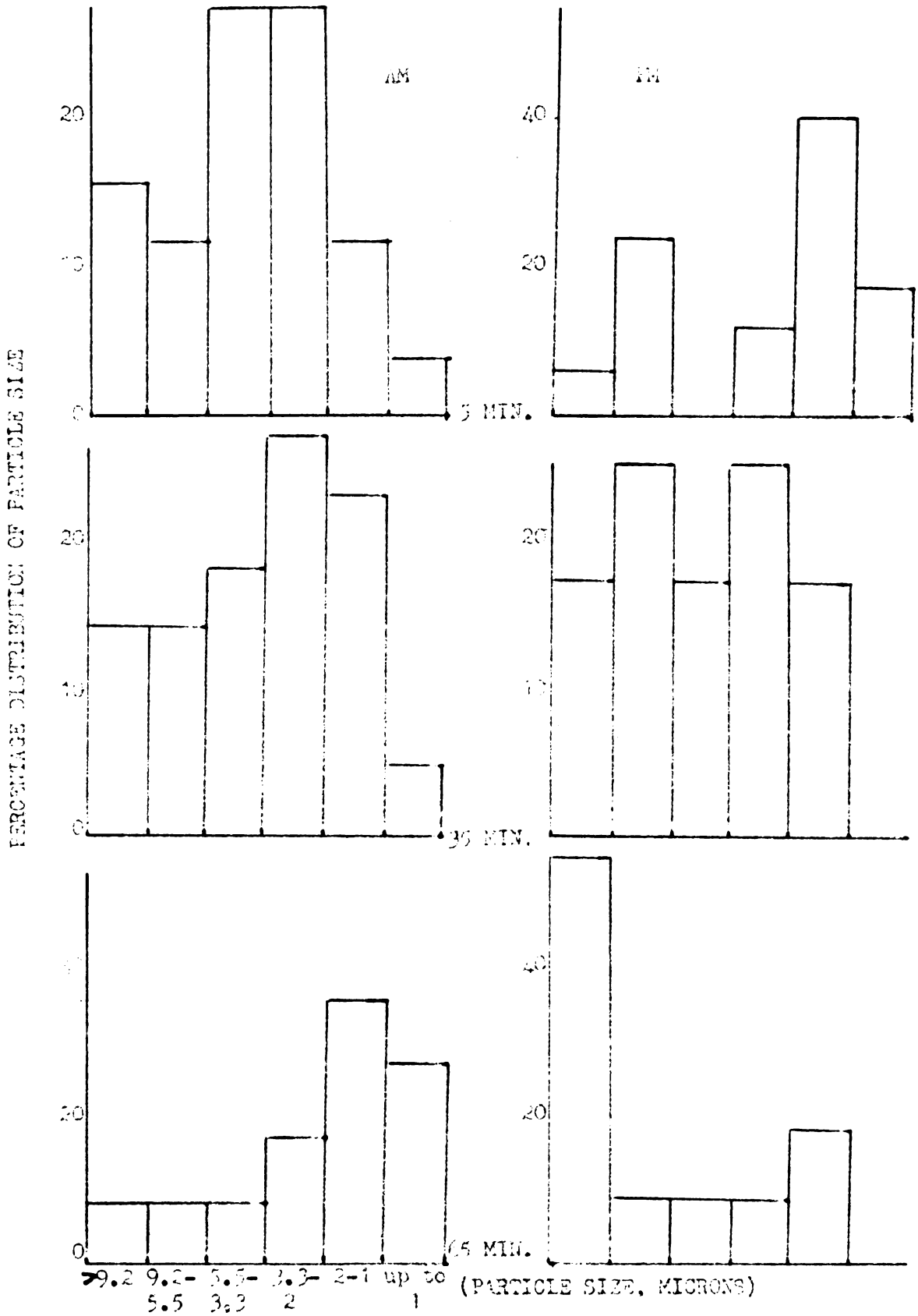
7. Particle size distribution of air-borne microorganisms shed by the untreated arm of Subject C



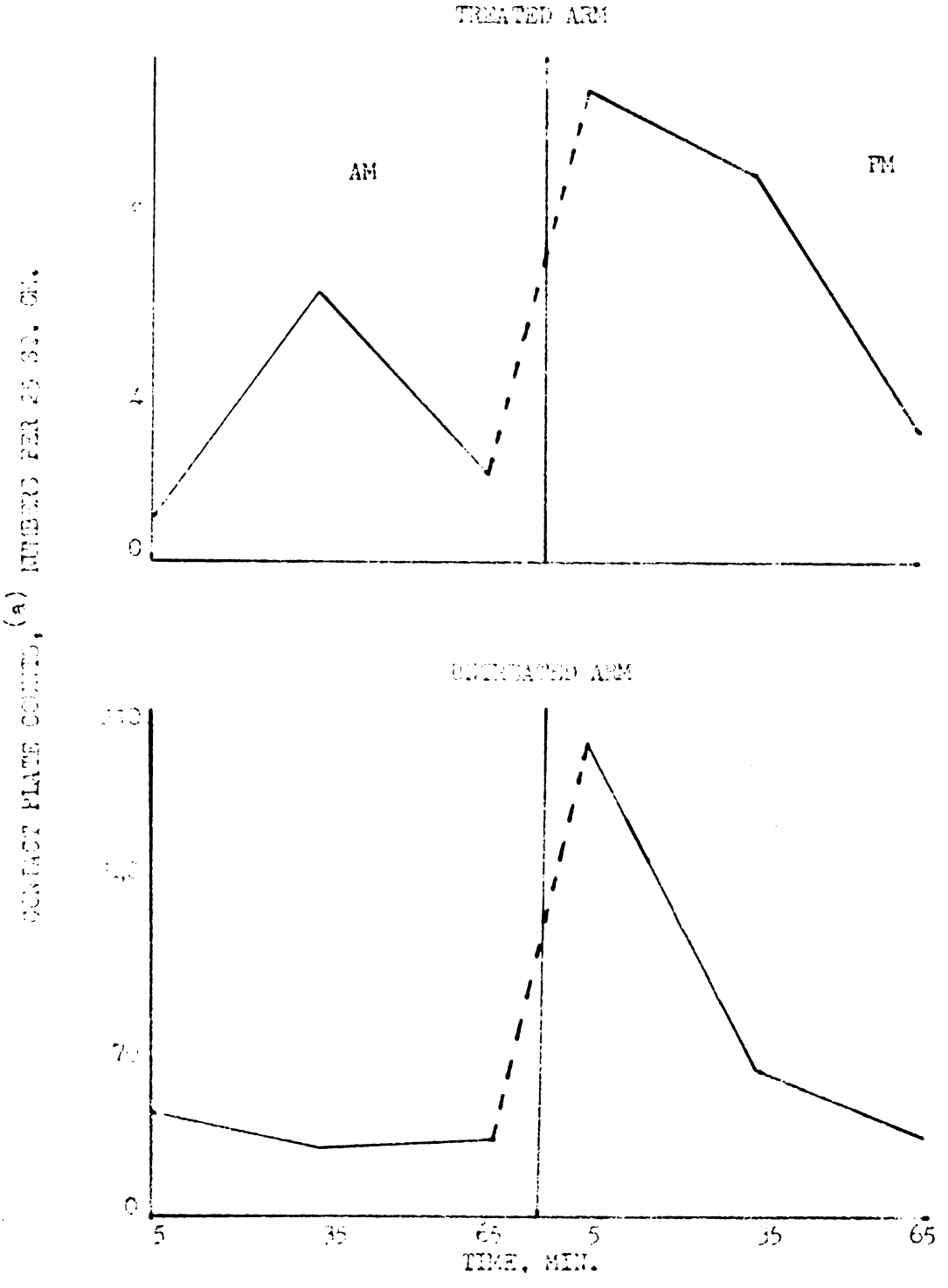
8. Particle size distribution of the air-borne microorganisms shed by the treated arm of Subject D



6. Particle size distribution of air-borne microorganisms shed by the untreated arm of Subject D

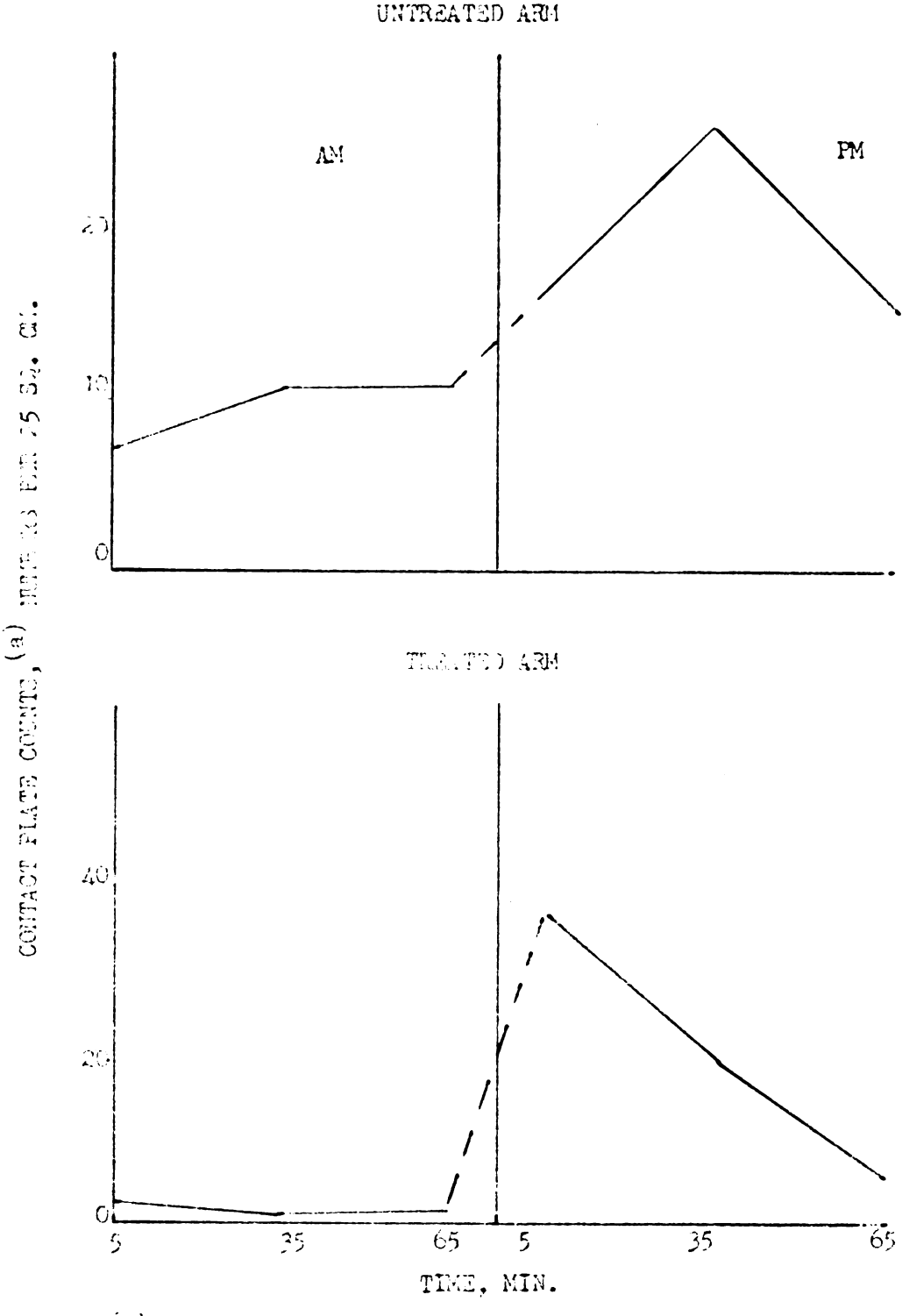


9. The relationship between vim; and surface plate count



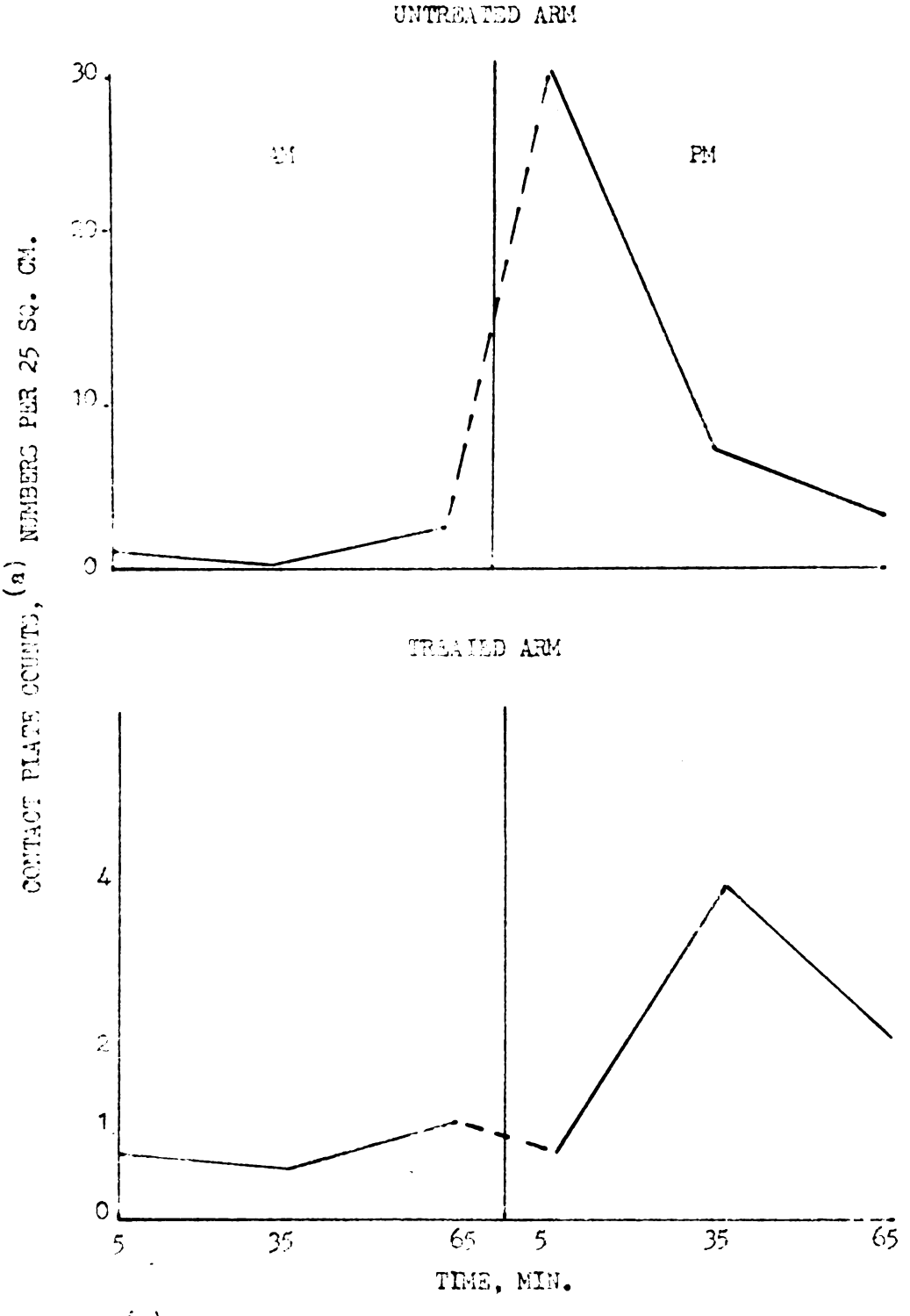
(a) Means of 12 observations.

10. The relationship between surface plate count and time for Subject A



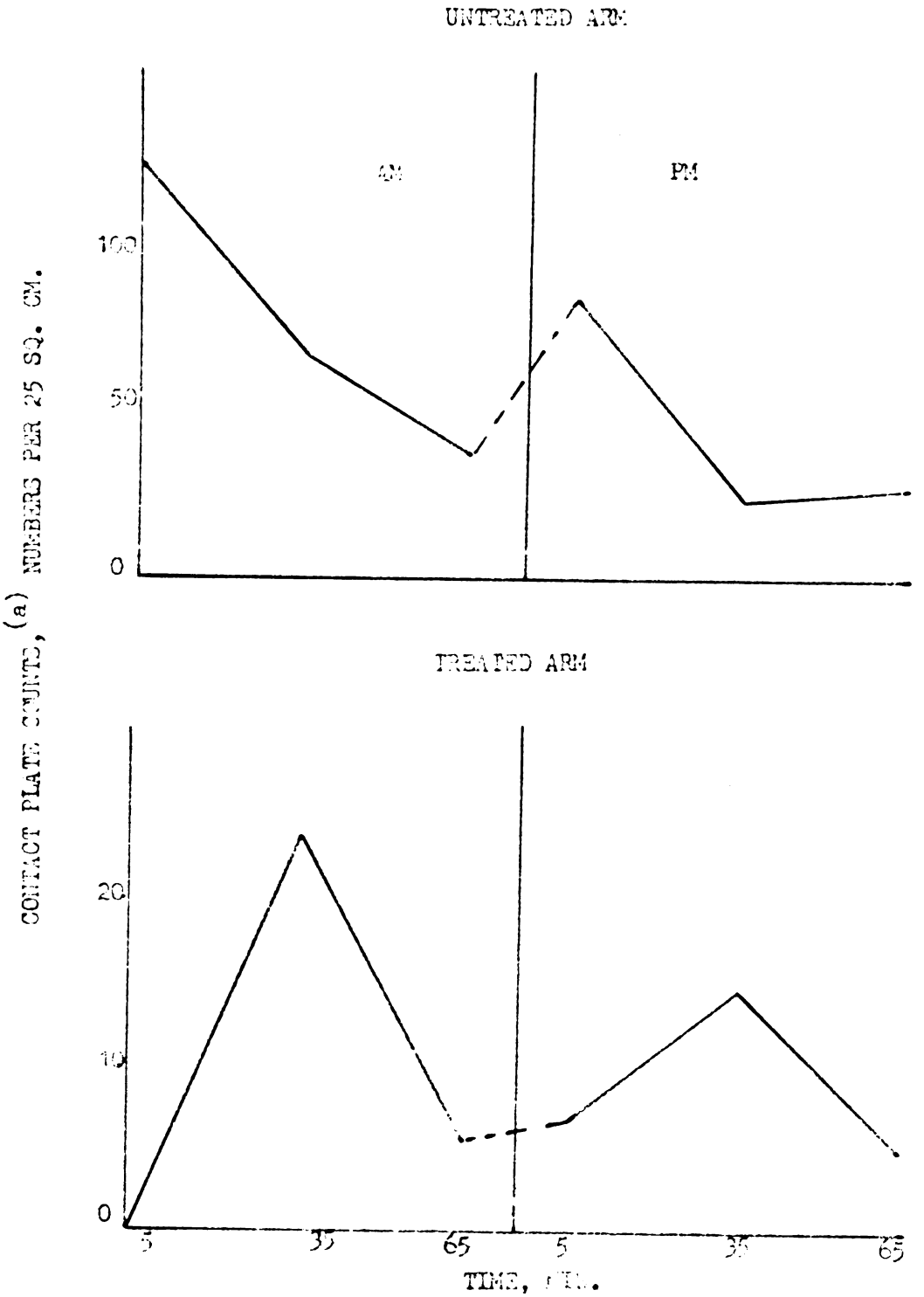
(a) Mean of five observations.

10. The relationship between surface plate count and time for Subject B



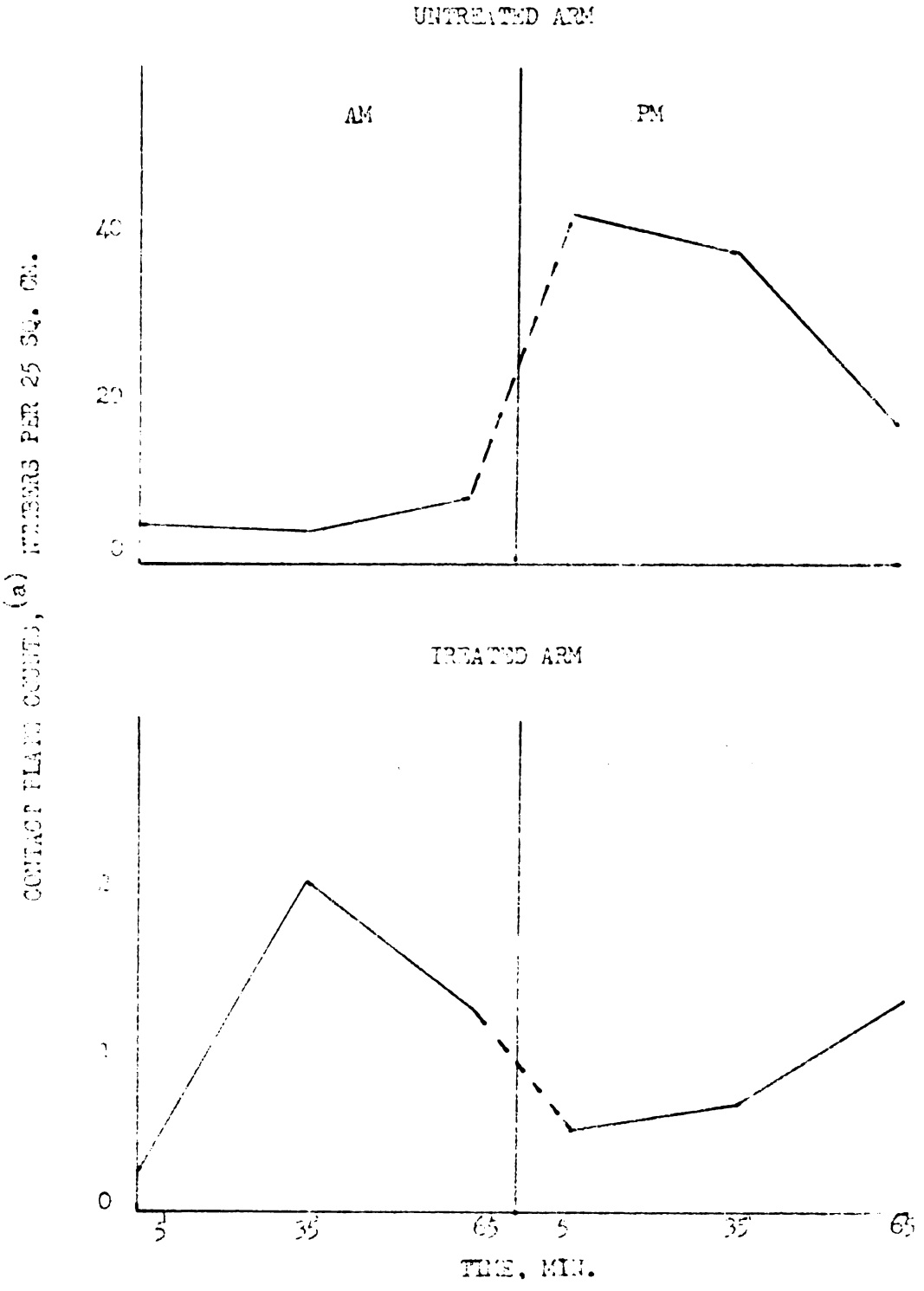
(a)Mean of five observations.

10. The relationship between surface plate count and time for Subject C



(a) Mean of five observations.

13. The relationship between surface plate count and time for Subject D



(a)Mean of five observations.

11. Source and time of isolation of the identified cocci

Subject	A				B				C				D			
Source of organism	TA		UTA		TA		UTA		TA		UTA		TA		UTA	
Sampling period	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
M. flavus				1												
M. luteus		1														
M. caseolyticus	1									1				1		
Sar. lutea	1														3	
Sar. hansenii	4	3	2			1								3	3	
Sar. aurantiaca	2		2						1	1	6	3	3	4	4	1
Sar. ventriculi										2	2	1				
Sar. flava	14	4	6	5	1	3	2	2			1					2
Pep. prevotii	3	4	2	7	3	2	1	1								5
Pep. saccharolyticus											1	1				
Staph. epidermidis				1	1							3	4	1	1	
Staph. aureus															1	
Gaff. tetragena						2	1									
Sub-total	25	12	12	14	5	8	4	3	1	4	10	8	7	9	12	8
Total	37		26		13		7		5		18		16		20	

12. Source and time of isolation of the identified rods

Subject	A				B				C				D			
Source of organism	TA		UTA		TA		UTA		TA		UTA		TA		UTA	
Sampling period	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Al. recti	1				1					1						
Al. marshallii	8	1	4		1	1		1		4	5		1	1		2
Al. bookeri	1	1	1	2				1	1	2	8	1		1		3
Al. metalcaligenes			1								1					
Al. viscolactis			1							2						
Al. faecalis			1	1												
Ps. mildenbergii	1			1							1					
Ps. iodinum	1	1	1					1	1					1		
Ps. aeruginosa	1		1						2							
Ps. fluorescens													2	1		
Ps. fragi								1			1					
Ps. synxantha										1	2	1	5	2		1
C. pyogenes	1		1													
C. acnes		1								1				1		
C. xerosis				1							2			1		
C. striatum											3					
Bac. firmus			1													
Bac. megaterium														1		
Sub-total	14	4	12	5	2	1	0	4	4	11	23	2	8	9	0	6
Total	18		17		3		4		15		25		17		6	

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