Possibility of *C. immitis* Infection of Museum Personnel

REYNOLD T. SCHMIDT, M.D., M.P.H., and DEXTER H. HOWARD, Ph.D.

IN November 1966 three members of an excavation team digging for fossil vertebrate remains near Maricopa, Calif., acquired acute coccidioidomycosis. The team was organized under the direction of the department of paleontology of the Los Angeles County Museum.

At that time a question was raised concerning the possibility of fomite transmission of coccidioidomycosis by fossils brought to the museum from the excavation site. All specimens brought from Maricopa after November 1966 were fumigated with methyl bromide gas, a standard procedure to prevent the introduction of insect pests into the museum. The specimens were exposed to methyl bromide (1.85×10^{-3} lbs./ft.^{3}) for 24 to 48 hours, but subsequent inquiry revealed that the ability of gas to sterilize them was unknown.

The purposes of this investigation were to (*a*) isolate *Coccidioides immitis* from the soil at the excavation site, (*b*) study the possibility of fomite transmission of coccidioidomycosis to museum personnel, (*c*) evaluate the antimicrobial effect of the methyl bromide fumigation procedure, and (*d*) make recommendations based on the results of the investigation.

History

The geographic distribution of *C. immitis* in certain parts of the world is well known. The fungus is found in the soil of the Lower Sonoran Life Zone in the southwest United States (*1*). In 1938 Dickson and Gifford (*2*) elucidated the essential clinical features of coccidioidomycosis from cases occurring among residents of the San Joaquin Valley of California, and this area is still among those with a high incidence of the disease.

The central valley of California has also long been an area of numerous paleontologic explorations. The north and east walls of the valley, formed by the Sierra Nevada Mountains, are poor in fossil remains, as they have been buried by silt from alluvial fans formed by the major rivers which originate there. However, vertebrate fossils have been abundant on the south and west sides of the valley. The first explorations of major dig sites in the valley date back to 1853 and 1865 (*3*, *4*).

Excavation at Maricopa. The first paleontologic deposit discovered at Maricopa several years ago (*5*) probably was an ancient waterhole near the foot of the mountains (fig. 1). The site differed little in the late Pleistocene from what can be seen today.

The basic objective of an excavation team is to obtain the fossil remains intact and to return them to the museum for identification and study. This objective is accomplished by carefully excavating all bones which can be removed easily from the soil (figs. 2, 3). The specimens which are resistant to gentle digging are circumvented, and blocks of soil containing the specimens are

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covered with a plaster cast (fig. 3). The blocks are removed to the museum where the bones are carefully extricated and separated (fig. 4).

The excavation site at Maricopa is unusual in that it is covered with a minimum of 2 feet of oil-impregnated sand, silt, and clay overburden (*5*). The bones are found in a mixture of clay and asphalt. The entire bone bed is covered with a 3-inch layer of fine dust from the surrounding area.

Specimens from other paleontologic excavation sites are stored in the Los Angeles County Museum, many of them in the original containers in which they were transported. In particular, specimens from Sharktooth Hill dig site near Bakersfield, Calif., and McKittrick dig site, 30 miles north of the Maricopa site in the southwestern portion of the San Joaquin Valley, are stored there. Investigators cultured *C. immitis* from the soil at Sharktooth Hill in 1956 (*1*).

The spores of *C. immitis* must be present in the soil at the Maricopa excavation site. It is postulated that the dust which carries the spores could collect on the surface of the plaster casts and be taken into the museum, exposing persons working with the casts, or those nearby, to the disease. Specimens from Sharktooth Hill and McKittrick dig sites already stored in the museum present a similar situation.

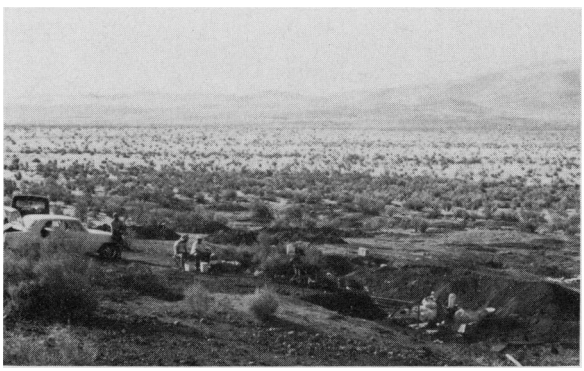

Figure 1. Maricopa excavation site

Figure 2. Plaster casts at dig site

Materials and Methods

Specimen collection. Soil and dust samples were collected in three different groupings and were cultured in the laboratory for *C. immitis*. The groupings were differentiated as follows.

1. Soil samples collected from the Maricopa excavation site and from rodent burrows within 100 yards of the site (fig. 5). All specimens were taken 4 to 6 inches beneath the surface.

2. Dust samples taken directly from depressions on the cast surfaces. These specimens were obtained before the casts were transported to the museum and from casts previously taken to the museum and which had not been subjected to methyl bromide fumigation. In addition, one cast was opened, and random samples were taken from within.

3. Dust samples collected from specimens taken in the museum from Sharktooth Hill and McKittrick dig sites and stored in the museum. These specimens had never been subjected to methyl bromide fumigation.

Sterilizing efficiency of methyl bromide. Plaster casts approximately 10 by 20 by 5 inches were constructed to resemble the large casts in every respect. Soil and bone specimens from the excavation site were used to duplicate as closely as possible the actual conditions. All the casts had soil samples in depressions on the external surfaces. The casts from each group were subjected to methyl bromide fumigation in the vacuum fumigator used by the Los Angeles County Museum. Bacterial and fungal colony counts were made on soil samples from within as well as on the surface of the casts. The casts were divided into two groups and were exposed to methyl bromide (1.85×10^{-3} lb./ft.^{3}) for 24 to 48 hours.

Isolation of C. immitis *from soil specimens.* One gram of each soil specimen was suspended in 20 ml. of 0.85 percent NaCl solution. The mixture was shaken vigorously with a stirrer for

1 minute and then the soil was allowed to settle for 20 minutes at room temperature. Five ml. of the supernatant were removed and treated with 10,000 units of penicillin and 2 mg. of streptomycin per ml.

One ml. of the treated supernatant was injected intraperitoneally into each of four mice per soil specimen. Four weeks after injection the mice were killed and small portions of their livers and spleens were inoculated onto tubes of Mycosal agar (BBL). The inoculated media were incubated at 30° C. for 6 weeks and were examined periodically for the development of *C. immitis*.

Routine quantitative plate counts for fungi and bacteria. One gram of the soil specimens was suspended in 10 ml. of 0.85 percent NaCl solution. Serial tenfold dilutions were prepared, and 0.1 ml. samples of the dilution were cultured on duplicate plates of glucose-peptone agar with 1,000 units of penicillin and 1 mg. streptomycin per ml. The glucose-peptone agar comprised 1 percent glucose, 2 percent Bacto proteose peptone No. 3 (Difco), and 2 percent agar. Other 0.1 ml. samples of the same dilution were cultured on duplicate plates of Bacto heart infusion agar (Difco).

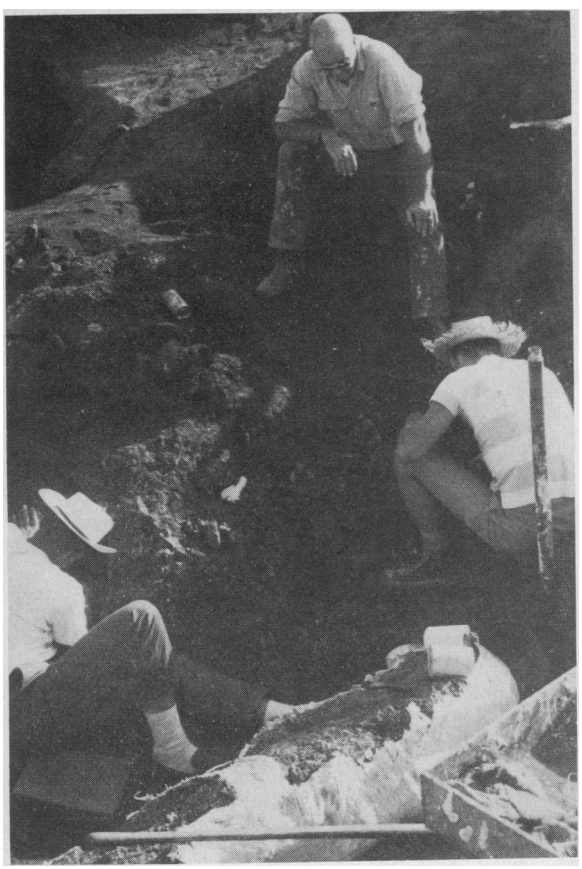

Figure 3. At work in the dig site

Figure 4. Opening the casts in the museum

The glucose-peptone agar plates were examined for fungus colonies after 1 week of incubation at 30° C. The heart infusion agar plates were examined for bacterial colonies after 24 hours of incubation at 30° C.

Results

C. immitis was isolated from one of 17 soil and dust samples from the Maricopa dig area. This isolate was obtained from a rodent burrow (fig. 5, rodent burrow 2A).

Spherules of *C. immitis* observed in a potassium hydroxide preparation from the lesions in a mouse injected with a suspension of soil from this site are pictured in figure 6. Figure 7 is a photomicrograph of a lactophenol cotton blue mount from the culture of the fungus grown from lesions of the same mouse. All of the eight soil or dust samples from materials stored at the Los Angeles County Museum were negative for *C. immitis* by the mouse inoculation technique.

Data from a study of the effect of methyl bromide fumigation on the resident microbial population of desert soils on or within simulated plaster casts are shown in the table. The methyl bromide procedure reduced the number of fungus colonies obtained from samples on the surface and from within the simulated plaster casts. *C. immitis* was not recovered in any of these direct plating procedures.

The occurrence of fungi in dirt from the surface of the cast and their absence or smaller numbers from the interior of the cast may have been due to the reintroduction of fungal spores into surface specimens by the air used to remove methyl bromide from the gasing chamber. This supposition was supported by the fact that no fungi were isolated from soil either on the surface or within the simulated casts when they were wrapped with a single layer of brown wrapping paper before fumigation and aeration.

The number of bacteria from soil specimens on the surface or from within simulated plaster casts was also reduced by the methyl bromide procedure. However, the specimens were not sterilized by the exposure to methyl bromide. Approximately a threefold to sixfold reduction in the number of bacterial colonies was observed (see table).

The difference in counts between the two time intervals and the location of the soil, that is, surface or interior of cast, probably reflect sampling errors. It is unlikely that these large

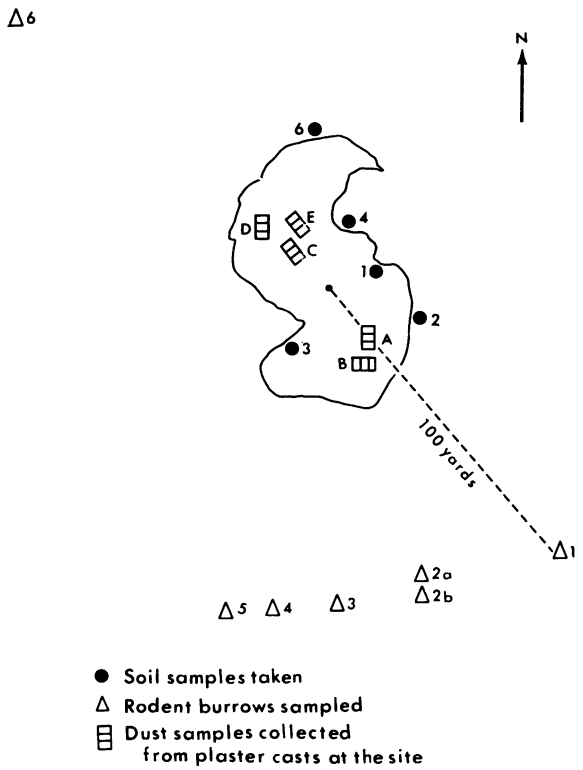

Figure 5. Sampling map of the Maricopa excavation site

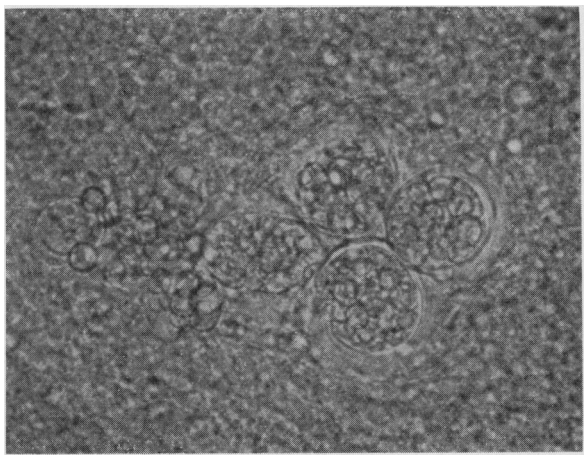

Figure 6. KOH mount of *Coccidioides immitis* spherules from lesions in mouse injected with soil sample (450X)

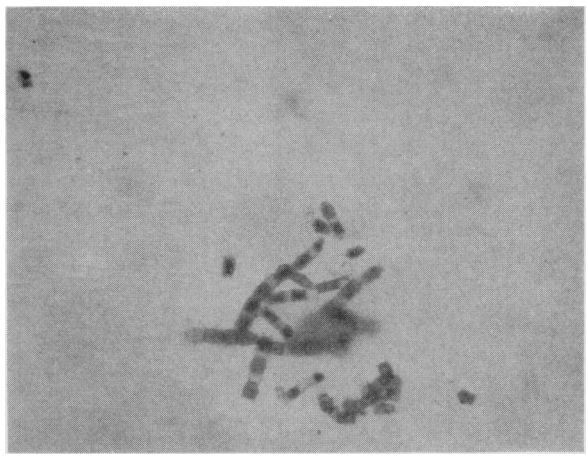

Figure 7. Lactophenol cotton blue mount from culture of fungus grown from *Coccidioides immitis* arthrospores in the lesions observed in mice (450X)

numbers of bacteria, approximately 5–9 x 10^5 bacteria per gram of soil, could have been introduced by the air used to rid the chamber of methyl bromide fumes. Furthermore, plaster casts wrapped in brown paper before fumigation and aeration provided essentially the same results as unwrapped casts with respect to the bacterial population.

Clearly, then, while methyl bromide reduced the resident microbial population of desert soil in or on simulated plaster casts, it did not sterilize the soil. Studies on the effect of methyl bromide on spores of *C. immitis* were not under-

taken because of the hazards involved in pursuit of such experiments under the conditions available to us.

Discussion

The dig site at Maricopa was excavated by 30 persons, most of them museum personnel and all of whom were white. It is of interest to note that the three persons who contracted acute coccidioidomycosis were volunteer workers. A mother and daughter, ages 45 and 13 years, and a 9-year-old boy, comprised the affected group. The woman and her daughter were not native Californians, but came to Los Angeles from Seattle, Wash., several years ago. The boy was born in Long Beach, Calif. These three persons took part in the initial digging at the site and were exposed to the surface layer of dust. Except for the stricken volunteers, only two members of the excavating team were skin test positive to coccidioidin after the episode.

Personnel from the department of paleontology at the Los Angeles County Museum have been involved intermittently for years in the excavation sites in the areas of the southwest in which *C. immitis* is endemic, yet they have never reported a case of active coccidioidomycosis. In addition, according to Dr. J. R. Macdonald, curator of vertebrate paleontology, Los Angeles County Museum, no museum personnel have ever contracted clinical coccidioidomycosis while working with specimens within the museum.

The excavation site is in the area where *C. immitis* is endemic (*1*). It would be realistic to assume that the possibility of contracting acute coccidioidomycosis is present, although the actual attack rate could not be predicted. The mode of work, including construction of plaster casts around the specimens, could promote transmission of spores of the causative organism to the museum.

Furthermore, the casts are sometimes left at the excavation site for 1 month before being transported to the museum. Dust blowing from areas around the site collects in the multiple depressions and crevices on the surfaces of the casts.

In addition, it was considered possible that spores of the organism might be on the soil blocks before they were casted. If this were true, personnel would be exposed to the hazard of spore inhalation as they opened the casts at the museum. It is important to note that the spores of *C. immitis* are resistant to desiccation and temperature changes and apparently can remain viable in soil samples for years (*1*).

Once the casts are taken to the museum, they are exposed for 24 to 48 hours to methyl bromide gas. The casts are opened with an electric cast opener such as orthopedic surgeons use. Personnel then work with sharp instruments, within 6 inches of the soil blocks, to separate and extricate individual bones.

Dust particles forced into the air by these procedures were not measured. However, no dust particles were visible during these operations. The specimens from the McKittrick and Sharktooth Hill excavation sites are stored in

Effect of methyl bromide fumigation on resident microbial population of desert soils on and in simulated plaster casts

Source of soil sample	Time exposed to methyl bromide gas (hours)	Number of fungus colonies at 10^{-1} dilution [1]		Number of bacterial colonies at 10^{-3} dilution	
		Plate 1	Plate 2	Plate 1	Plate 2
Maricopa dig site	0	9	6	235	255
Surface of cast	24	[2] 3	2	60	48
Interior of cast	24	0	1	93	95
Surface of cast	48	3	1	88	90
Interior of cast	48	0	0	55	35

[1] Lowest practicable dilution employed. Data presented as counts on duplicate plates.
[2] The apparent discrepancy between colony counts of surface and interior soils probably is related to possibility of reintroduction of fungus spores while ridding the chamber of gas fumes.

open wood boxes, just as they were brought from the sites.

The transmission of coccidioidomycosis by fomites has been well documented (*6*). Most of the proved cases were due to transmission of the fomite of spore-containing dust from an endemic area. Although it is possible for a person to acquire a localized cutaneous granuloma by inoculation of the organism, most cases are thought to occur secondary to inhalation of the spores.

The greatest number of soil specimens positive for *C. immitis* can be obtained during spring and fall (*7*). The organism is killed by temperatures greater than 125° F. (*6*). In addition, an increased rate of recovery of the organism occurs when soil samples are obtained from rodent burrows (*1*). The soil samples for this investigation were obtained during the summer of 1967. At this time of year soil temperatures at the surface can exceed 140° F., which may partly explain the low yield of positive cultures. The soil temperatures would also be a reason for the negative results from the samples of dust from the casts. However, the principle of fomite transmission is still valid. Also, the total of 17 samples cultured is small in comparison with the total area of the Maricopa site.

The fumigation tank used at the Los Angeles County Museum is of the vacuum type, 72 inches wide and 15 feet long. One pound of methyl bromide is used per load. Casts from the excavation site are fumigated at room temperature only after being punctured to allow for internal circulation of the gas. Experimental data show that sterilization was not obtained, even after 48 hours' exposure to methyl bromide. The gas apparently did have a fungicidal action, at least as measured by the decrease in the total number of colonies. Whether the gas will kill the spores of *C. immitis* was not tested.

Conclusions and Recommendations

Although this investigation failed to demonstrate the existence of fomite transmission of coccidioidomycosis into the Los Angeles County Museum, the principle of fomite transmission of this disease is established (*6*). Future excavations by museum personnel might well raise the same questions again.

Because the present method of using methyl bromide gas does not sterilize the specimens, we suggest that an ethylene oxide-halogenated hydrocarbon mixture be used in the vacuum sterilizer. This gas has been proved effective in killing both the saprophytic and parasitic forms of *C. immitis* (*8*).

An important factor to consider is the toxicity of the method used. The threshold limit value of methyl bromide is 20 ppm, as opposed to 50 ppm for ethylene oxide (*9*). Data quoted by Stierli and co-workers (*8*) indicate that a person can work in an atmosphere containing 250 ppm ethylene oxide in single exposures of 1 hour without being harmed. Thus, the ethylene oxide gas would be a safer, as well as a more effective means of sterilization.

An effort to prevent primary infection of personnel at the excavation site must be made. Consideration must be given to typical environmental conditions at most excavation sites. The sites are often located in remote areas, and the excavating usually is done during the summer months. High temperatures and the nature of the work probably would preclude wearing of masks. The excavation site and the surrounding area might be dampened with water in an effort to control the dust.

Recently an experimental fungicide, 1-chloro-2-nitropropane, has been shown to be effective against *C. immitis* in commercially feasible dilutions and quantities (*10*). This fungicide penetrates the soil sufficiently for partial and temporary control of the fungus within 24 hours of spraying, is equally effective at 25° C. and 40° C., and leaves no contaminating soil residue. In addition, this fungicide could be sprayed on the outside of the plaster casts before they are transported to the museum. These procedures might substantially reduce the possibility of fomite transmission.

Additional features of a control program for limiting the incidence of occupational coccidioidomycosis and reducing the severity of the disease in those who become infected have been outlined by Schmelzer and Tabershaw (*11*).

Summary

After three members of an excavation team acquired acute coccidioidomycosis in November

1966, all specimens brought from the Maricopa, Calif., dig site to the Los Angeles County Museum were fumigated with methyl bromide gas from 24 to 48 hours.

Because the effectiveness of the gas was unknown, an investigation was undertaken to isolate *Coccidioides immitis* from the soil at the excavation site, study the possibility of fomite transmission of coccidioidomycosis to museum personnel, evaluate the antimicrobial effect of methyl bromide fumigation, and make recommendations based on the results of the investigation.

Soil and dust samples from the site were collected in three different groups and cultured for *C. immitis* in the laboratory. Small facsimiles of casts for containing bone specimens transported from the excavation site were subjected to methyl bromide fumigation and various laboratory tests.

C. immitis was isolated from one of 17 soil and dust samples from the Maricopa dig area. This isolate was obtained from a rodent burrow. Spherules of *C. immitis* were observed also in a potassium hydroxide preparation from the lesion in a mouse injected with a suspension of soil from the site. All eight soil or dust samples from materials stored at the Los Angeles County Museum were negative for *C. immitis* by the mouse inoculation test.

Methyl bromide fumigation reduced the number of fungus colonies from samples on the surface and from inside the simulated casts. *C. immitis* was not recovered in any of the direct plating procedures. No fungi were isolated from soil either on the surface or inside the simulated casts wrapped in a single layer of brown paper before fumigation and aeration.

While methyl bromide reduced the resident microbial population of desert soil in or on the simulated casts, it did not sterilize the soil. Approximately a threefold to sixfold reduction in the number of bacterial colonies was observed. The gas apparently did have fungicidal properties, at least as measured by the decrease in the total number of colonies. Whether the gas will kill the spores of *C. immitis* was not tested.

It was suggested that an ethylene oxide-halogenated hydrocarbon be used for fumigating the specimens because it has been proved effective in killing saprophytic and parasitic forms of *C. immitis*, and the hydrocarbon would be safer for persons working with it.

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phr synopses

BLOCH, DORIS (University of California School of Public Health, Berkeley): *Role change of public health nurses working with indigenous aides. Public Health Reports, Vol. 83, October 1968, pp. 811–819.*

A small, exploratory study was done in a large county health department in California which had started to use indigenous aides in the public health nursing program. Ten public health nurses were interviewed to elicit their feelings about the function of the aides and about their own changing roles.

In this early stage of the program the role of the aides was not spelled out explicitly and the nurses were free to experiment with the role of their aides.

Functions of aides were classified into five groups: nonprofessional, subprofessional, nontraditional, health teaching, and new career tasks.

Most nurses reported that they used their aides for some of the nonprofessional tasks, as well as for simple subprofessional and nontraditional tasks. However, tasks involving health teaching were infrequently turned over to the aides, and few of the nurses seemed committed to turning new career-type tasks over to their aides.

Almost all the nurses saw their role as changing from one with an emphasis on patient contact to one of supervision and teaching. Their reaction to this change was not favorable, since they liked patient contact. They felt that supervising an aide was time consuming, and they were unhappy about the clerical work involved. They reported some difficulty in finding enough work for the aides to do, possibly because they were uncomfortable about the reallocation of functions.

Generally, their feelings about this new program were mixed, but they were making an effort to adjust to their new roles. All felt that indigenous workers should not be judged as a group, but that they are individuals with different assets. They saw a great need for more careful selection, orientation, and training of indigenous workers.

HARTMANN, FLOYD W. (California State Department of Public Health), THOMAS, JUNE, and HOKOM, LESTER: *The microbiological quality of selected food products. Public Health Reports, Vol. 83, October 1968, pp. 873–881.*

Commercially produced egg salad sandwiches, chicken salad sandwiches, banana cream pies, and custard pies were subjected to bacteriological testing by selected local health departments in California. Bacteriological procedures used were the standard plate count (S.P.C.) and coliform plate count (coliform count).

The correlation between S.P.C. and coliform count was high for three of the four foods studied. This indicates that one type of count might suffice for similar work in the future. The S.P.C. was consistently higher than the coliform count, usually by a factor of 100. Counts for egg salad sandwiches and banana cream pies were slightly higher than chicken salad sandwich counts. Custard pie counts were lowest. The number of organisms per gram of food varied widely—the S.P.C. range was from less than 100 to more than 1 billion per gram; the range for the coliform count was from less than 10 to more than 100 million.

Significant differences in bacteriological counts were apparent among the four geographic areas studied. There are many variables which might account for the differences, but it was not possible to extricate and identify them. Although there was a tendency for foods with lower temperatures at time of collection to show lower counts, there was no strong association of these two variables. The data from this survey do not show that the numbers of organisms vary in all instances with such factors as prepackaging, season, type of retail establishment, or time between preparation of food and delivery to the retail establishment.

Counts differed significantly among the various production plants. Further work is needed to ascertain the bacteriological quality which could be expected if all producers and distributors of a given food were employing maximum sanitary control. Thorough sanitary surveys, coupled with bacteriological studies, are required to define what constitutes good commercial practice.

phr synopses

PARRY, HUGH J. (George Washington University): *Use of psychotropic drugs by U.S. adults. Public Health Reports, Vol. 83, October 1968, pp. 799–810.*

Evidence from two current surveys of national samples suggests that about one of four U.S. adults uses one or more kinds of psychotropic drugs. Nearly half the U.S. adult population report the use of a psychotropic drug at some time. Stimulants are used by the smallest proportion, sedatives by a larger proportion, and tranquilizers by the largest group. Cumulative use of tranquilizers over a decade has shown a steady increase—from about 7 percent of the population in 1957 to about 27 percent in 1967.

There are relatively few significant differences in prevalence of use by major demographic groupings. Major differences appear to be related to sex, religion, and race. Women are markedly higher in use than men; Jews are higher than Protestants or Catholics in overall use and in sedatives and tranquilizers, but not in stimulants. Lower proportions of Negroes than of whites use these drugs; the pattern for both sexes among Negroes is fairly similar to that for white men. Among whites, in contrast, there are fairly large differences between the sexes. The two groups with high prevalence of psychotropic drug use (women and Jews) have low rates of escape drinking; the group with low prevalence (Negroes) displays high escape drinking rates.

Higher income seems to be associated with higher use in the Northeast and North Central regions, but not in the South and West. People apparently tend to adopt the drug use patterns of the economic groups that they are moving up or down into. The use by men in the highest income bracket differs only slightly from the use by men in the lowest; for women, the differences by income level are more substantial.

SCHMIDT, REYNOLD T. (University of California, Los Angeles) and HOWARD, DEXTER H.: *Possibility of* C. immitis *infection of museum personnel. Public Health Reports, Vol. 83, October 1968, pp. 882–888.*

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phr synopses

GILBERT, ARNOLD (Mount Zion Hospital and Medical Center, San Francisco), and O'ROURKE, PAUL F.: *Effects of rural poverty on the health of California's farmworkers. Public Health Reports, Vol. 83, October 1968, pp. 827–839.*

Poverty is nearly twice as prevalent in rural California as in metropolitan areas, and urban areas oriented toward agriculture have twice the poverty of economically diversified communities.

The ethnic breakdown of farm laborers in California probably includes 60 percent Mexican-Americans, 30 percent Anglo-Americans, and 10 percent Negroes. Nearly 65 percent of their homes are substandard—10 percent have no water supply, and pit privies service one-third. Average quarterly wages in 1963 ranged from $417 to $449.

These workers have a low rate of hospital admissions and medically attended conditions, despite high rates of bacterial and parasitic infections, circulatory disease, and accidents. Agriculture has the highest occupational disease rates and the second highest rate of disabling work injuries of any major industry in California. In 1962, 10 representative agricultural counties with a combined population of 2,438,000 had only 131.3 physicians per 100,000 persons.

Despite continuing inadequacies of medical service delivery, clinics initiated under nongovernmental as well as governmental auspices are providing improved medical services for farmworkers in some counties. In 1966, 11 of 17 counties with migrant health projects had clinics for the workers.

Prospects for overcoming the financial barriers to medical care and the shortages of health manpower and facilities in rural areas depend largely on the achievement by the majority of farmworkers of collective bargaining with health benefits as part of their contracts with growers.

In the meantime the Federal Government could join with medical societies and local health departments in rural areas to develop projects staffed by Public Health Service physicians. Development of such projects might be combined with scholarships for medical students committed to practice for specified periods in rural areas, resident and intern rotations to rural hospitals, and rural preceptor programs for medical students. A crucial factor in the potential for success of such activities is the willingness of medical schools to accept community service training as an integral part of their teaching responsibilities.

GRUENWALD, PETER (Sinai Hospital of Baltimore, Inc.): *Fetal growth as an indicator of socioeconomic change. Public Health Reports, Vol. 83, October 1968, pp. 867–872.*

Fetal growth may be studied in populations by means of birth weight curves, relating weight to gestational age. Comparison of data obtained in different groups or at different times is a useful indicator of socioeconomic differences or changes. Theoretical considerations of fetal growth during the third trimester indicate that in most instances data on pregnancies lasting less than 35 weeks from the last menstrual period, which are difficult to obtain in significant numbers, need not be considered.

Sample standards, based on a voluntary hospital in Baltimore, are given as well as extrapolated standards indicating how a population of fetuses would presumably grow if optimally supplied. The extrapolated standards should be useful in comparing populations from different parts of the world. Studies of fetal growth may help in the future to pinpoint significant factors within the broad spectrum of socioeconomic conditions.

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U.S. GOVERNMENT PRINTING OFFICE: 1968