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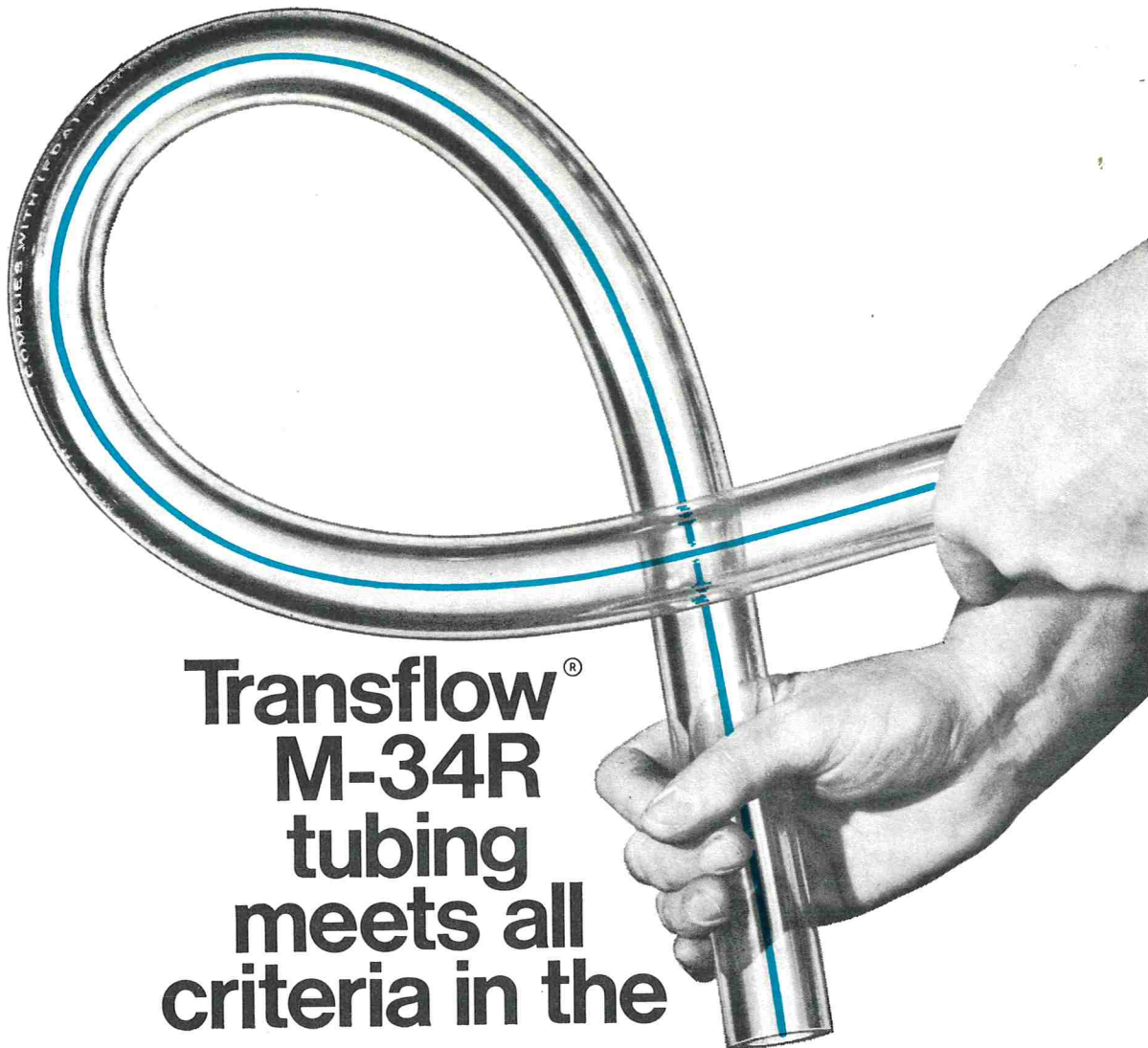
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PUBLIC HEALTH PROBLEMS ASSOCIATED WITH BARBECUED FOOD. A REVIEW

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(Received for publication June 22, 1972)

ABSTRACT

Barbecued foods are popular ready-to-eat products which are increasing in sales in many countries throughout the world. They are sold from grocery stores, restaurants, take-out shops, and outdoor stalls. These foods, mainly barbecued chicken, have caused 62 known outbreaks of food poisoning, some of them involving hundreds of people and causing deaths. Although freshly barbecued foods, adequately cooked, are wholesome and contain few bacteria, subsequent unsanitary handling and storage in inadequate holding facilities increases the risk of bacterial contamination; these bacteria may grow to hazardous levels. Stored barbecued chickens have been found with high bacterial counts. Temperature control of barbecued food is considered the best way of preventing food poisoning. Unfortunately, legislation in most countries does not specify actual temperature requirements for storing of potentially hazardous foods, which includes barbecued products. We recommend that barbecued food should be stored at ≤ 40 F or ≥ 140 F.

Barbecuing must be one of the oldest forms of cooking meat, ever since primitive man spit-roasted birds and other game over an outdoor fire. Yet commercial barbecuing is only a relatively recent venture, beginning in the heavily populated areas of North America and extending to Europe, Australia, and to a certain extent the Far East. Barbecued foods, mainly meats, are normally sold from restaurants, take-out restaurants, and supermarkets. The last two types of establishments, particularly supermarkets, cater to an ever-increasing demand for ready-to-eat food by the public, and it is from these that most barbecued meats are sold. In addition, large-scale organized picnics involving many hundreds or thousands of people frequently have barbecued food on the menu, with a caterer cooking on the site or delivering the cooked food to the picnic area. Along with increased consumption, increased numbers of microbial food poisoning outbreaks have occurred. Some of these outbreaks were relatively small, but others have incapacitated several hundred people.

The private outdoor barbecue is also popular in North America during the summer months. There is unlikely to be a health problem from barbecuing done by individual families because the food is usually eaten as soon as it is cooked. However, day-long picnics for organizations, where large amounts

of food are barbecued by amateur cooks, can cause outbreaks (64).

As expansion of commercial barbecuing outlets continues, dangers of food poisoning to the public continue to rise. The purpose of this review is to outline the world-wide demand for barbecued food, to summarize known food-poisoning outbreaks, and to report on existing legislation dealing with conditions for holding barbecued food pending sale and overnight storage.

ESTIMATION OF THE SALES VOLUME OF BARBECUED FOODS

In response to enquiries we received information on the sales volume of barbecued foods from 80 countries, 10 provinces (Canada), 52 states (United States of America and Australia), and 5 territories, districts, etc. The information we received is variable in nature and it is not possible to compare the situation from one country to another with great accuracy. Nevertheless, the general demand for barbecued foods throughout the world can be presented.

Canada

The demand in Canada, mainly from Retail Food Establishments, is moderate to large in all provinces except Newfoundland. In the more northerly regions, naturally, opportunities for barbecuing outlets are limited by the small and scattered population.

In the National Capital Region, including the cities of Ottawa and Hull, there are 52 Retail Food Establishments selling an estimated 15,000 barbecued chickens per week or about 1.5 per capita per year (E. Todd, unpublished work).

United States of America

In the United States of America the demand for barbecued foods is large or increasing in over one-half of the states. Many areas have almost reached saturation level with rotisseries in almost every supermarket. Finite data of sales are not readily available from most states. However, an estimate from Georgia (78), where sales of barbecued foods are not considered to be large, indicates that there are rotisseries in 30-40% of Retail Food Establishments (a

total of 800 rotisseries); these produce about 86,000 chickens per week (78) or about 1.0 per capita per year (E. Todd, *unpublished work*).

Whereas all states have Retail Food Establishments selling barbecued food, only 29 of the states have Food Service Establishments (restaurants, snack bars, etc.) regularly serving this food. In some states Food Service Establishments rarely retail barbecued products; this may be explained by the following information from R. K. Clary (12): in Wisconsin very few restaurants serve barbecued chickens because the staff cannot produce the volume required for restaurant operation, and poultry sold at take-out restaurants is usually deep-fat fried or pressure cooked.

Caribbean

In the Virgin Islands (US) and in Trinidad and Tobago the demand is great from restaurants and take-out restaurants; in the Virgin Islands there are also large sales from supermarkets. In Cuba, Dominica, St. Lucia, St. Vincent, Barbados, and Grenada the demand is small or non-existent.

Central America

There is a large demand in Mexico from stalls, small shops, and some supermarkets, and in Costa Rica from restaurants. There is a small volume of sales in El Salvador, Guatemala, Nicaragua, Panama, and Honduras, mainly from restaurants and take-out restaurants.

South America

Venezuela and Peru have a large demand from restaurants, and in Venezuela also from supermarkets, but in Argentina, Bolivia, and Chile barbecued food is less popular and is sold mainly in restaurants. The demand in Uruguay is small and seasonal, being confined mainly to seaside resorts. In Guyana this kind of food is rarely consumed.

Africa

There is almost no demand in most of the African countries.

Europe

In Europe the demand for barbecued food is generally increasing but nowhere, with the exception of some parts of England, does it equal that of the large cities of the west and east coasts of the United States. There is a relatively large and increasing demand in England, Ireland, France, Norway, Sweden, Denmark, Finland, Austria, Switzerland, Yugoslavia, and Greece; all these countries sell barbecued food from take-out restaurants and supermarkets, the exceptions being Norway and Greece where barbecued food is offered at restaurants and take-out restaurants only. There is a moderate demand in Belgium, Portugal, and Hungary;

the first two countries retail the products in supermarkets and take-out restaurants, Hungary only from take-out restaurants. In Scotland and Poland, where the demand is small, barbecued food is sold only from supermarkets; in the Netherlands and Romania where the demand is also small, restaurants are the sole source of barbecued food. In some ways it is surprising that the Netherlands has such a small demand for barbecued food, as this country is one of the most progressive in the marketing of hot ready-to-eat foods from vending machines. In Czechoslovakia and the USSR barbecued food is virtually non-existent. Barbecued chicken is apparently a very popular snack item for tourists in some of the Mediterranean countries (e.g., Spain and Italy) especially at coastal resorts.

Asia

In the Middle East, Israel has a small but increasing demand from supermarkets, take-out restaurants and snack bars; Cyprus, Iran, Iraq, and Kuwait have a limited demand for western-style barbecued food, but consumption of barbecued kebabs of chicken and lamb produced in stalls and small shops is great. In Afganistan, India, Ceylon, Pakistan, and Nepal there is almost no demand, but farther east there are considerable sales from supermarkets and take-out restaurants in Thailand and to a lesser extent in Singapore. In Japan, Taiwan, Hong Kong, and the Philippines there is a small but, in many areas, increasing demand, mainly from restaurants and shops.

Oceania

In New Zealand barbecued food is sold in supermarkets and take-out restaurants, but its popularity has declined since its introduction in 1958, because of the growing demand for a less expensive product — fish and chips. In Australia, although there are no figures for consumption of barbecued chickens they are, probably, proportional to the general poultry consumption which has risen from 13 lb. per capita in 1966 to 20 lb. per capita in 1970. Each of the states, including Tasmania, has a large and increasing demand for barbecued food sold in supermarkets, departmental stores, and take-out restaurants; only in Victoria do supermarkets not play the major role in marketing this kind of food. The demand is small in the sparsely populated Northern Territory of Australia. In Hawaii, one of the United States of America, the demand is large with some 40 establishments, both supermarkets and restaurants, selling barbecued foods.

In summary, many countries of the world sell barbecued food on a commercial basis. The demand is large or increasing in 25% of the countries where there is some estimate of the popularity of this kind of ready-to-eat food.

Types of barbecued foods

Barbecued chicken is, with few exceptions, the most popular of barbecued foods anywhere in the world where rotisseries are installed. Greece is one of the few countries where the demand for barbecued chicken is surpassed by another barbecued product—veal; similarly barbecued sausages are more popular than barbecued chickens in Poland. In all other countries other barbecued food ranks low in priority after chicken, although there are available barbecued turkey, pork, ham, spare ribs, and less frequently beef, lamb, and sausage in North America; sheep and pork in Mexico; beef in Argentina; turkey in England; pork in Greece and the Phillipines; kasseler (lightly smoked pork loin), beef, and spare ribs in Sweden; duck and pork in Singapore and duck, frankfurters, hamburgers, shishebabs and potatoes in Australia.

FOOD POISONING

International distribution of outbreaks

We know of food-poisoning outbreaks arising from consumption of barbecued food in five countries that have large outlets for this kind of food. There is little doubt, however, that many outbreaks pass undocumented as food poisoning is not a notifiable disease in most countries. In addition, few food-poisoning outbreaks are reported in established journals; most come from personal communications or from health reports or bulletins with limited distribution (Table 1). This makes the collection of information about outbreaks difficult, and the 62 outbreaks from barbecued food recorded in Table 1 are probably a fraction of those that have occurred.

Of these 62 outbreaks, 47 occurred in the United States of America, 9 in England, 3 in Canada, 2 in Australia, and 1 in Sweden. Information, though not always complete, was published in about two-thirds of the outbreaks; we learned of the remainder through personal communications. It is natural that the United States with its large population and large consumption should lead the field with the number of outbreaks; innovation in large-scale production and marketing may have contributed to these. Yet many states do not report food-poisoning incidents and many more outbreaks no doubt occur. England and Wales have an advanced system for reporting food-poisoning outbreaks and probably all large food-poisoning outbreaks occurring there in the last decade have been documented. One would expect other European countries with populations similar to, or greater than that in England, and with comparable consumption of barbecued food to have a similar or greater number of outbreaks. However, to our knowledge, only one outbreak, in Sweden, has been recorded on the continent.

Numbers of outbreaks

Table 2 shows the annual total of outbreaks from before 1962 until 1970. There appear to be some isolated outbreaks before 1966, a peak from 1966 to 1968, and a decrease thereafter. The peak is caused mainly by an increase in the number of outbreaks due to barbecued chicken, reflecting the rapid increase in popularity of this food, especially in supermarkets, in North America and England. The decrease in number of outbreaks from 1969 to 1970 is probably an indication that many food-poisoning episodes occurring during these years have not yet been publicized. For example, the food-poisoning figures for England and Wales, 1968, were published in 1970 (88). Therefore, it is likely, that the number of outbreaks for 1969 and 1970 was greater than appears in Table 2.

The numbers of cases in the food-poisoning outbreaks range from one to over a thousand with an average of about 80 cases per outbreak, a relatively high figure. There were 18 outbreaks with 1-10 cases, 16 with 11-100, 8 with 101-1000, and one outbreak with over a thousand cases.

Sources of outbreaks

Table 3 lists the sources of the barbecued products which were involved in the outbreaks. Retail Food Establishments supplied the food for 25, more than one-half of the outbreaks of known source. Food Service Establishments and catering firms each accounted for 7 outbreaks, although the average number of victims per outbreak is greater from the latter.

Causes of outbreaks

Table 4 lists the causes of outbreaks. All causes can be included in two categories: (a) insufficiently barbecued meat where food-poisoning bacteria survived, or (b) adequately cooked meat which was subsequently contaminated by food-poisoning bacteria. It is likely that in most cases the cooked meat was stored at temperatures which would allow growth of these bacteria.

Insufficient thawing of frozen meat without an increase in cooking time or temperature may lead to survival of bacteria in the centre of a large mass of meat, such as a turkey, and has caused several outbreaks (3, 30, 56, 65, 71). Inadequate cooking may also result from ignorance of the time-temperature combination or from defective rotisseries. Eleven outbreaks are associated with inadequate cooking (Tables 1 and 4).

Raw meat, in particular poultry, is known to contain salmonellae and other food-poisoning bacteria before cooking (7, 79, 94). These pathogens can be transferred to cooked meat, either by direct contact with the infected raw meat, or by surfaces contaminated by the raw meat, e.g., hands, utensils and

TABLE I. FOOD-POISONING OUTBREAKS AND SINGLE CASES FROM BARBECUED FOOD

| Out-break No. | Barbecued food involved | Location | Year of outbreak | Source of barbecued food | Food-poisoning organism | Cause of outbreak | No. of cases | Reference |
|---------------|-------------------------|--------------------------------------|------------------|---|---|---|-------------------|-----------|
| 1 | Chicken | North Carolina, USA | 1958 | Pit barbecue associated with school cafeteria | <i>Salmonella</i> ² <i>typhimurium</i> | Probable recontamination of cooked chickens from raw chickens | Ca. 170 | (25) |
| 2 | Chicken, beef and pork | Covington, Louisiana, USA | 1962 | Home | <i>Salmonella</i> ^{1, 2} <i>typhimurium</i> | Unknown | 12 | (10) |
| 3 | Chicken? | Sedgwick Co., Kansas, USA | 1963 | Unknown | <i>Salmonella</i> ² <i>oranienburg</i> | Unknown; implicated with other food but no food cultured | 10 | (28) |
| 4 | Chicken | Adelaide, South Australia, Australia | 1965 | Take-out restaurant | <i>Staphylococcus</i> ^{1, 3} <i>aureus</i> | Insufficiently thawed and inadequately cooked; cooked birds stored at room temperature | Ca. 120 | (4) |
| 5 | Chicken | Spokane, Washington, USA | 1966 | Supermarket | <i>Salmonella</i> ^{1, 2, 3} <i>typhimurium</i> | Barbecuing (140 F) and storage (100 F) temperatures not hot enough. <i>Salmonella</i> could survive cooking and grow subsequently | 107 (2 deaths) | (2, 91) |
| 6-14 | Chicken | Los Angeles Co., California, USA | 1966-67 | Unknown | <i>Salmonella</i> sp., <i>Staphylococcus aureus</i> or <i>Clostridium perfringens</i> | Unknown | 9* | (72) |
| 15 | Chicken | Humble, Harris Co., Texas, USA | 1967 | "Purchased" | <i>Salmonella</i> ¹ <i>javiana</i> | Unknown | 4 | (40) |
| 16 | Chicken | Ottawa, Canada | 1967 | Supermarket | <i>Staphylococcus</i> ¹ <i>aureus</i> | Storage temperature probably not hot enough and the bacteria could multiply (chickens purchased 6 months after outbreak were at 110 F) | 1 | (59) |
| 17 | Chicken | Bolton, England | 1967 | 1 Supermarket and 2 chicken retailers | <i>Salmonella</i> ^{2, 3} <i>virchow</i> | Staff, raw chickens and equipment all shown to be contaminated after outbreak; cooked chickens probably recontaminated from raw birds and equipment | 22 | (35) |
| 18 | Chicken | Portsmouth, England | 1968 | Garden barbecue | <i>Salmonella</i> ^{1, 2} <i>panama</i> | Probably insufficiently thawed and inadequately cooked; recontamination also likely | 32 | (56, 57) |

| | | | | | | | | |
|----|---------|--------------------------------|------|---|--|--|----------|----------|
| 19 | Chicken | Liverpool, England | 1968 | Cooked meat shop catering for tennis club social, restaurants | <i>Salmonella</i> ^{1, 2, 3} <i>virchow</i> | Shop and restaurants supplied from same contaminated packing station; chickens not thawed adequately before cooking; barbecuing not hot enough to kill the <i>Salmonella</i> and subsequent quartering, packaging and storing at about 65 F allowed growth of bacteria | 162 | (71) |
| 20 | Chicken | England | 1968 | Market | <i>Salmonella</i> ¹ <i>virchow</i> | Unknown | 2 | (89) |
| 21 | Chicken | Bath, England | 1968 | Restaurant | <i>Salmonella</i> ^{1, 3} <i>montevideo</i> | Chickens insufficiently thawed out and undercooked; raw birds stored with cooked birds in same refrigerator | 40 | (30, 89) |
| 22 | Chicken | Winchester, England | 1968 | Shop | <i>Salmonella</i> ^{1, 2, 3} <i>montevideo</i> | Undercooking owing to defective rotisserie | 22 | (32, 92) |
| 23 | Chicken | England | 1968 | Shop | <i>Salmonella</i> ^{1, 3} <i>montevideo</i> | Unknown | 19 | (89) |
| 24 | Chicken | England | 1968 | Supermarkets and fish-mongers | <i>Salmonella</i> ¹ <i>montevideo</i> <i>Salmonella</i> ¹ <i>panama</i> | Unknown Unknown | 47 16 | (89) |
| 25 | Chicken | England | 1968 | Unknown | <i>Salmonella</i> ¹ <i>enteritidis</i> var. <i>jena</i> | Unknown | 8 | (89) |
| 26 | Chicken | Stockholm, Sweden | 1968 | Supermarket | <i>Salmonella</i> ^{1, 2, 3} <i>thompson</i> | Recontamination of cooked chickens by raw chickens and personnel; storage not hot enough (86-104 F) | 212 | (39) |
| 27 | Chicken | New South Wales, Australia | 1968 | Catering firm | <i>Salmonella</i> sp. | Barbecued chickens left unrefrigerated for several hours | 111 | (18) |
| 28 | Chicken | McKinleyville, California, USA | 1968 | Food store | <i>Staphylococcus</i> ¹ <i>aureus</i> | Unknown | 3 | (46) |
| 29 | Chicken | Rhode Island, USA | 1969 | Supermarket | <i>Salmonella</i> ² <i>heidelberg</i> | Undercooked; storage temperature not hot enough (110 F) | 3 | (67) |
| 30 | Chicken | Tuscola Co., Michigan, USA | 1969 | Food store | <i>Salmonella</i> ^{1, 2} <i>berta</i> | Insufficiently cooked and/or contaminated after cooking; held at 100 F in store and kept 1-4 hr at ambient temperature by customers | 24 | (43, 44) |
| 31 | Chicken | Delaware, USA | 1970 | Supermarket | "paracolon ¹ bacilli coli- form group" | Improper cooking and storage temperature (110 F) allowed bacterial growth | 1 | (29) |

| | | | | | | | | |
|-------|--------------------|--|---------|--|--|--|-----------------------|----------|
| 32 | Chicken | Victoria, British Columbia, Canada | 1970 | Supermarket | <i>Staphylococcus</i> ^{1, 2} <i>aureus</i> | Unknown, though storage temperatures were unsafe | 5 | (49) |
| 33 | Turkey | Port Orchard, Washington, USA | 1962 | Home | <i>Salmonella</i> ² <i>typhimurium</i> | Undercooking and subsequent holding of turkey at room temperature for 3 days resulted in growth of bacteria | 10 | (1) |
| 34 | Turkey | Monterey Co., California, USA | 1964 | Home | <i>Salmonella</i> ^{1, 2} <i>newport</i> ; <i>S.</i> <i>anatum</i> ^{1, 2} , <i>S. heidelberg</i> ^{1, 2} <i>S. blockley</i> ^{1, 2} | Probable undercooking and growth of bacteria during storage of cooked turkey at room temperature for one day | 8 | (47, 48) |
| 35 | Turkey | Los Angeles Co., California, USA | 1966-67 | Unknown | <i>Salmonella</i> sp., <i>Staphylococcus</i> <i>aureus</i> , or <i>Clostridium</i> <i>perfringens</i> | Unknown | 1* | (72) |
| 36 | Turkey | Oxford, Nebraska, USA | 1967 | Barbecue pit for town festival | <i>Salmonella</i> ^{1, 2} <i>typhimurium</i> | Turkey rolls inadequately thawed and insufficiently cooked; cutting boards contaminated | Ca. 1350 (1 death) | (64, 65) |
| 37 | Pork sandwiches | Northern New Jersey, USA | 1963 | Sandwich shop | <i>Salmonella</i> ^{1, 2, 3} <i>heidelberg</i> | Pork contaminated after barbecuing during chopping on unclean board; incubation for several hours at room temperature allowed bacteria to grow | Ca. 100 | (26) |
| 38 | Pork | Wake Co., North Carolina, USA | 1964 | Barbecue establishment | <i>Salmonella</i> ^{1, 2} <i>infantis</i> | Recontamination of cooked meat by raw meat through mishandling by employee | 54 | (31, 37) |
| 39-41 | Pork | Los Angeles Co., California, USA | 1966-67 | Unknown | <i>Salmonella</i> sp., <i>Staphylococcus</i> <i>aureus</i> , or <i>Clostridium</i> <i>perfringens</i> | Unknown | 3* | (72) |
| 42 | Pork | Henry Co., Tennessee, USA | 1967 | Take-out restaurant | <i>Salmonella</i> ^{1, 2, 3} <i>chester</i> | Opportunity for contamination of cooked pork by poor handling; storage temperature (52 F) not cold enough | Ca. 90 | (84) |
| 43 | Pork | Bleckley Co., Georgia, USA | 1967 | Open barbecue pit associated with a sand- wich shop | <i>Salmonella</i> ^{2, 3} <i>typhimurium</i> | Cooked pork probably contamin- ated by raw pork or food handler; preparation and storage conditions suitable for growth of bacteria | 10 | (45) |
| 44 | Pork | Selma, Dallas Co., Alabama, USA | 1969 | Take-out restaurant | <i>Staphylococcus</i> ^{1, 3} <i>aureus</i> | Cooked pork probably contamin- ated by food handler; probably inadequate refrigeration allowed bacteria to grow | 10 | (14) |

| | | | | | | | | |
|----|------|---|------|---|--|---|---------|----------|
| 45 | Pork | Hampton, South Carolina, USA | 1969 | Barbecue pit associated with a restaurant | <i>Staphylococcus aureus</i> ¹ | Barbecued pork stored at room temperature for 6-10 hr; equipment unsanitary | Ca. 40 | (83) |
| 46 | Pork | Nashville, Tennessee, USA | 1969 | Raw pork barbecued at an establishment; taken to caterer where cut up and warmed for community picnic | <i>Staphylococcus aureus</i> ^{1, 3} | Food handler probably contaminated pork after initial cooking and prior to final warming; cooked pork kept at ambient temperature overnight | Ca. 320 | (70, 86) |
| 47 | Pork | Memphis, Tennessee, USA | 1969 | Open pit associated with a restaurant | <i>Staphylococcus aureus</i> ^{1, 3} | Excessive preparation time without refrigeration; probable contamination from food handlers; sandwiches stored at temperatures suitable for bacterial growth | 93 | (63, 85) |
| 48 | Pork | Clarksville, Montgomery Co., Tennessee, USA | 1970 | Open pits associated with a restaurant | <i>Salmonella thompson</i> ^{1, 2, 3} | Food handlers probably recontaminated cooked meat; storage temperatures for raw and cooked meat not adequate to prevent growth of bacteria; sanitation poor | 303 | (24, 93) |
| 49 | Pork | Iredell Co., North Carolina, USA | 1970 | Restaurant | <i>Salmonella typhimurium</i> ^{1, 2, 3} | Pork was not adequately barbecued and food handlers became infected; ham subsequently contaminated by handlers; inadequate refrigeration of ham and barbecued pork resulted in outbreak | 56 | (41) |
| 50 | Pork | Jackson, Tennessee, USA | 1970 | Restaurant | <i>Salmonella typhimurium</i> ^{1, 2, 3} | The cutting board was contaminated from raw pork and recontaminated the barbecued pork | 63 | (51, 52) |
| 51 | Ham | Shreveport, Louisiana, USA | 1962 | Catering firm for picnic | <i>Salmonella newport</i> ^{1, 2} | Unknown | 32 | (87) |
| 52 | Ham | East Providence, Rhode Island, USA | 1967 | Supermarket | <i>Staphylococcus aureus</i> ¹ | Unknown | 6 | (66) |
| 53 | Ham | Mountain View, California, USA | 1968 | Restaurant | <i>Staphylococcus aureus</i> ² | Cooked ham kept at 66 F until completely sliced for sandwiches, and any bacteria present would multiply | 2 | (36) |

| | | | | | | | | |
|-------|---------------------------|----------------------------------|---------|------------------------|---|---|----|---------|
| 54-57 | Spare ribs | Los Angeles Co., California, USA | 1966-67 | Unknown | <i>Salmonella</i> sp., <i>Staphylococcus aureus</i> , or <i>Clostridium perfringens</i> | Unknown | 4* | (72) |
| 58 | Beef | New York City? USA | 1947? | Delicatessen | <i>Streptococcus</i> ¹ <i>faecalis</i> | Cooked meat left unrefrigerated for 16 hr before reheating slightly gave ample opportunity for bacterial growth | 74 | (8) |
| 59 | Beef | Dallas Co., Texas, USA | 1962 | Take-out restaurant | <i>Salmonella</i> ^{1, 2, 3} <i>braenderup</i> | Possible cross-contamination from raw chickens to beef; possible contamination from mouse, since droppings found contained <i>S. braenderup</i> | 12 | (58) |
| 60 | Beef, chicken and sausage | Dallas Co., Texas, USA | 1963 | 2 Take-out restaurants | <i>Salmonella</i> ^{1, 3} <i>anatum</i> | Unknown | 9 | (58) |
| 61 | Lamb | Bridgewater, Nova Scotia, Canada | 1967 | Supermarket | <i>Salmonella</i> ^{1, 2, 3} <i>thompson</i> | Contamination from food handlers; storage temperature was ambient after cooking | 7 | (6, 13) |
| 62 | Laulau ⁴ | Hawaii | 1968 | Food store | <i>Salmonella</i> ^{1, 2} <i>give</i> | Probable recontamination from raw pork; storage temperature (97-125 F) not enough to prevent growth of bacteria | 2 | (38) |

¹Isolated from suspected food.²Isolated from food-poisoning victims.³Isolated from food handlers.⁴Made from pork wrapped in taro and ti leaves and steamed; the hot product is stored as for barbecued food (packaged and kept in a warm display cabinet).

*Outbreaks and single cases; number of people not stated.

Ca. = Approximately.

trays. This probably explains why recontamination of cooked products from raw meat may be one of the most frequent reasons for the presence of food-poisoning bacteria in barbecued foods (61, 72, 82). Many outbreaks have arisen from this source of contamination: in barbecued chicken (25, 30, 35, 39, 57, 71), turkey (65), pork (24, 31, 38, 45, 83, 84), and beef (58).

Another major source of contamination of barbecued food is infected food handlers. These handlers may be symptomless excretors of salmonellae or may be colonized in the anterior nares with enterotoxigenic staphylococci. These bacteria may contaminate the cooked food during handling. Infected food handlers have been involved in 8 outbreaks from barbecued chicken, 9 from pork, 2 from beef, and 1 from lamb (Table 1).

In addition to a source of contamination an incubation period is usually required for the contaminants to grow before a food-poisoning outbreak can occur. In 24 of the 62 outbreaks, barbecued food was known to have been stored at temperatures which would allow food-poisoning bacteria to grow. No doubt these conditions occurred in other outbreaks.

Examples of outbreaks

In the following section four outbreaks not previously published are described in detail:

(a) *Barbecued chicken outbreak in Sweden* (39). On February 11, 1968, 12 of a party of 24 people fell ill after having eaten barbecued chickens at a snack bar in a supermarket the previous day. A further 200 cases were reported during the next few weeks in Stockholm and its vicinity; all had consumed barbecued food at the same snack bar. The clinical symptoms were typical of salmonellosis—a 24 hr incubation period, fever, vomiting, and diarrhea. *Salmonella thompson* was isolated from the victims, barbecued chicken, barbecued kasseler, barbecued spare-ribs, and from cutting boards used for preparing the meat in the snack bar, but not from raw chickens; 25 of 370 employees in the supermarket were carriers of *S. thompson*. Barbecued meats were stored on heated open display tables at 86-104 F, an ideal temperature range for growth of salmonellae. It seems likely that some raw chicken or other raw meat contained low numbers of *S. thompson* and these infected some of the food handlers and contaminated the cutting boards. Subsequent batches of chicken, which may have been free from salmonellae, were barbecued and then contaminated by food handlers and the cutting boards. Incubation of the barbecued food on display tables allowed rapid growth of the salmonellae, which in turn caused a large outbreak of food poisoning. The result of this outbreak and similar ones stimulated the Veterinary Board, which is responsible for regu-

lating the handling of foodstuffs in Sweden, to issue special regulations on March 6, 1968 for barbecued food (see Legislation Section).

(b) *Barbecued pork outbreak in South Carolina* (83). On July 4, 1969 pork had been cooked in a barbecue pit associated with a snack bar. The pit racks and covering were in an unsanitary condition and impossible to clean and disinfect satisfactorily. Meat not sold that day was held at room temperature 4-6 hr and then put in large enamel dishpans and placed in an overloaded freezer. Six hogs were barbecued on July 9, and this barbecued pork was thoroughly mixed with the pork of July 4 after the latter had been thawed at room temperature. This mixture was allowed to remain at room temperature for 2-4 hr before being placed unheated on the serving line for buffet-type service. On July 10, 40 persons eating the barbecued pork for lunch at the snack bar fell ill and 12 of these were hospitalized. On investigation it was found that most of the ill persons had a short incubation period of a few hours, symptoms of nausea, diarrhea, and vomiting, but little or no pyrexia. Food preparation surfaces were cracked and in poor repair. The barbecued pork had a standard plate count of 2.0×10^7 per gram, a coagulase-positive staphylococcus count of 7.0×10^6 , and a coliform count of 1.7×10^5 . The presence of staphylococci and coliforms in the meat indicated contamination after cooking through improper food handling technique. The employees, when asked, had no idea of the significance of storing potentially hazardous food outside the safe temperature range (≤ 45 or ≥ 140 F in South Carolina regulations). It was concluded that the illness was caused by staphylococcal food poisoning with the pork probably being contaminated by staphylococci from one of the food handlers. This outbreak shows inadequate training of food handlers and the difficulty of enforcing temperature regulations.

(c) *Barbecued lamb outbreak in Nova Scotia* (6, 13). At 4:30 pm on October 14, 1967 a family from Bridgewater, Nova Scotia, purchased barbecued lamb from a food store, and ate it at 6:00 pm the same day. About 8 hr later all members of the family consisting of 2 adults and 5 children became acutely ill with diarrhea and vomiting. *Salmonella thompson* was isolated from barbecued lamb that had not been eaten, from stools of 6 members of the family, and from 3 of the 5 food handlers in the store and 3 of their family contacts. After the lamb had been barbecued it was placed in a warming cabinet that apparently was not heated or heated only slightly; according to the family the meat was still warm on purchase. The family did not refrigerate the lamb before consumption. From these items of information it seems certain that the lamb was at a suitable tem-

TABLE 2. ANNUAL TOTALS OF NUMBER OF OUTBREAKS AND SINGLE CASES INVOLVING BARBECUED FOOD

| Barbecued food | Before 1962 | 1962 | 1963 | 1964 | 1965 | 1966 ¹ | 1967 ¹ | 1968 | 1969 | 1970 |
|----------------|-------------|------|------|------|------|-------------------|-------------------|------|------|------|
| Chicken | 1 | 1 | 1 | — | 1 | 5 | 8 | 11 | 2 | 2 |
| Turkey | — | 1 | — | 1 | — | 1 | 1 | — | — | — |
| Pork | — | — | 1 | 1 | — | 2 | 3 | — | 4 | 3 |
| Ham | — | 1 | — | — | — | — | 1 | 1 | — | — |
| Beef | 1 | 1 | 1 | — | — | — | — | — | — | — |
| Lamb | — | — | — | — | — | — | 1 | — | — | — |
| Others | — | — | — | — | — | 2 | 2 | 1 | — | — |
| Total | 2 | 4 | 3 | 2 | 1 | 10 | 16 | 13 | 6 | 5 |

¹The 17 outbreaks and single cases reported by Senn and Williams (72) took place in 1966-1967; 9 of these were arbitrarily assigned to 1966 and 8 to 1967.

TABLE 3. FOOD SOURCES INVOLVED IN OUTBREAKS FROM BARBECUED FOOD

| | Retail Food Establishments ¹ | Food Service Establishments ² | Catering firms for social events | Home | Unknown |
|---|---|--|----------------------------------|------|---------|
| Number of outbreaks | 25 | 7 | 7 | 3 | 20 |
| Average number of victims per outbreak ³ | 39 | 85 | 311 | 10 | — |

¹Supermarkets, grocery stores, retail meat shops, delicatessens, take-out restaurants, etc.

²Restaurants.

³Overall average for all known sources = 85.

TABLE 4. SUMMARY OF CAUSES OF OUTBREAKS¹

| Bacterial growth associated with inadequate cooking when using: | | Bacterial growth associated with barbecued meat | | | | Unsafe storage temperatures | Unknown |
|---|----------------------------|---|---|--|----|-----------------------------|---------|
| Frozen or partially frozen meat | Fresh or refrigerated meat | Recontamination from raw meat | Cross-contamination from different material | Contamination from infected food handler | | | |
| 5 | 11 | 14 | 5 | 20 | 24 | 29 | |

¹Many of the outbreaks could be attributed to several possible causes; see Table 1.

TABLE 5. KNOWN SURVEYS OF THE RETAILING OF BARBECUED FOOD

| Survey | Country | Location | Retail outlet | Reference |
|--------|-----------|---|---|-----------|
| I | USA | Los Angeles County | Not stated | (72) |
| II | Canada | Vancouver, Winnipeg, Toronto, Montreal, Halifax | Grocery stores | (61) |
| III | Canada | Vancouver district | Grocery stores, butcher shops and delicatessens | (68) |
| IV | Canada | Vancouver district | Grocery stores, butcher shops and delicatessens | (69) |
| V | Canada | Ottawa-Hull district | Grocery stores | (82) |
| VI | Australia | Melbourne | Grocery stores and delicatessens | (77) |
| VII | Australia | Brisbane | Grocery stores | (55) |

perature for rapid growth of *Salmonella* for some hours between barbecuing and consumption. Contamination of the lamb most likely came from one or more of the infected food handlers. How the food handlers originally became infected however, is not known. As a result of this confirmed outbreak the 3 infected store employees were not allowed to handle

food until they stopped excreting salmonellae, and barbecued lamb was no longer put on sale. However, despite cooperation of the store staff with public health officials in bringing the outbreak under control, instruction in food storage still seems necessary; when the warming cabinet was examined 3 months after the outbreak it was at 120 F, well be-

TABLE 6. THE RETAILING PROCEDURE FOR BARBECUED CHICKENS ON DAYS AFTER OVERNIGHT STORAGE

| Retailing procedure | Surveys | | | | | |
|--|-------------------|------|------|------|-------|------|
| | I | III | IV | V | VI | VII |
| Refrigerated until sold | 20.0 ¹ | 61.1 | 60.0 | 56.9 | 0.0 | 28.6 |
| Left at ambient temperature | 0.0 | | | 4.8 | 0.0 | 0.0 |
| Put in hot-holding facilities | 34.3 | 16.7 | 11.1 | 31.3 | 0.0 | 28.6 |
| Reheated and put in hot-holding facilities | 31.4 | 22.2 | 15.6 | 1.0 | 0.0 | 14.2 |
| Not resold ² | 14.3 | 0.0 | 13.3 | 2.0 | 100.0 | 28.6 |

¹Percent of retail establishments.

²Includes food discarded or cut up for sandwiches, etc.

low the safe limit.

(d) *Laulau outbreak in Hawaii* (38). Laulau is a traditional Hawaiian food made from pork wrapped in taro leaves with ti leaf covering the whole product, and steamed. Taro (*Colocasia esculentia*) is a plant of the arum family with starchy leaves and rootstock which become edible after boiling and steaming. The ti plant (*Cordyline terminalis*) is of the lily family and the large leaves are used for wrapping food. Thus, although the food is not barbecued but steamed, the necessity of storing it hot is the same as for barbecued food. This was the case in an outbreak that occurred in Honolulu in 1968. On October 25, two of a family of 5 ate commercially packaged laulau purchased from a food store. Between 12 and 18 hr after consuming the food these two persons suffered headache, nausea, abdominal cramps, fever, diarrhea, and body ache. *Salmonella give* was isolated from these ill persons, remnants of the food in a polyethylene bag, and from a caecal swab of a hog from the laulau processor's farm. *Salmonella livingstone* was also found in the juices of the laulau.

Laulau, packaged in polyethylene bags, was held pending sale on a table heated by infrared lamps. Temperatures of the packages on display after the outbreak were 97 F at the bottom, 115 F at the middle and 125 F at the top. These temperatures are outside those recommended by Hawaii Department of Health (≤ 50 or ≥ 140 F) and show the inadequacy of heating food on open tables with infrared lamps. It would appear that the *Salmonella* contaminated the pork at the laulau processing establishment, either by survival in the steamed laulau, or more likely as a result of chance recontamination of the cooked laulau by pig excreta (as only one outbreak occurred and two *Salmonella* were involved); the food handlers themselves were not excreting *Salmonella*. Once contamination had taken place the incubating temperature of the display area permitted growth of the *Salmonella*. The interesting

point of this outbreak is that business interests took a traditional local dish and sold it as a commercial ready-to-eat product. This commercialization of laulau is comparable to the rise of the barbecue chicken industry; in each case the transition period from domestic preparation to commercial production is accompanied by public health hazards.

SURVEYS OF RETAILING PRACTICES

A limited number of surveys of retailing practices of barbecued food has been carried out. The surveys known to us mostly relate to barbecued chickens sold from supermarkets. There are two categories of survey; one which is based on questionnaires and inspections, the other on microbiological analysis of the chickens.

Assessment by questionnaires and inspections

Seven surveys are listed in Table 5 and are referred to in the following sections by Roman numerals.

Raw poultry. Chickens were usually stored refrigerated or packed in ice, but some chickens were frozen (III). Barbecuing of frozen chickens may result in inadequate cooking, and some outbreaks of food poisoning have been ascribed to use of unthawed poultry; bacteria probably survived the cooking process (4, 5, 57, 71).

Frequency of cooking. Surveys III, IV, and V showed that many outlets cooked the chickens two to three times a day, mostly to meet rush hour demand, and up to a third of the outlets barbecued all day to keep up with the demand. The remaining surveys did not state the frequency of cooking.

Barbecuing. Oven temperatures were regulated up to 500 F with a median of about 350 F. Chickens were barbecued for indefinite periods of time and were normally tested for "doneness" by twisting a leg or prodding with a fork or skewer.

Some chickens were considered to be inadequately cooked (< 190 F in III and IV) when inspectors

measured temperatures of the thigh muscles of barbecued birds. However, the temperature of the meat regarded as adequate at completion of cooking varies from >165 F (91) to 195 F (16). Unfortunately, even the manufacturers of barbecue equipment may fail to state the temperature required in the finished product, although they do recommend using a meat thermometer (42). In practice, very few rotisseries were found to have thermometers (V, VII) for measuring the temperature of the chickens.

Handling of cooked meat. The handling of the meat from the spit to the package is the most likely stage for microbial contamination of the cooked product. Spits containing chickens were removed from the rotisseries and placed on trays, counters, or paper-covered wooden meat blocks. Forks, tongs, wooden utensils, and gloved or bare hands were used to transfer the poultry into the packaging material. Only three surveys gave any information on the sanitation of handling. In one the hygiene was found to be satisfactory (VI), but in the other two it was inadequate. In V, only 17.3% of the stores said that they disinfected tools and trays before or after use, and about one-third (38.5%) stated that the same tools and trays were used for both raw and cooked meat; about two-thirds of personnel questioned were unaware of bacterial recontamination from raw to cooked meat.

The chickens were usually packaged in unsealed aluminum foil-laminate bags (I, III, V, VI, and VII), but I and V reported that aluminum trays and plio-film packaging were also used.

Holding facilities. Three main types of holding facilities were used to display the barbecued food at ready-to-eat temperatures: (a) heated enclosed cabinets; (b) open tables, cabinets or counters, heated from below or above, or both; and (c) unheated tables, cabinets, or counters. The temperatures of these holding facilities varied considerably from survey to survey, and temperatures inadequate for safe holding (<140 -143 F) of the poultry varied from 25% (III) to 74% (II). Few of the holding facilities contained thermometers (V, VII).

Storage practices. All surveys reported that at least some barbecued food was stored overnight, usually under refrigeration. However, a few retailers held packages at ambient temperature, left them in the warming cabinet, or discarded them. The procedure for the following day, however, presented no set pattern. The results are shown in Table 6. About 60% of the outlets in Canada refrigerated chickens until sold, often at a reduced price, but only 20% in Los Angeles County, California and 28.6% in Brisbane did so. Relatively few outlets reheated the stored barbecued food before placing it into hot-holding facilities; often this food is placed

directly from the refrigerator into the facilities. It would thus take a long time for the food to reach the upper safe temperature limit. This probably accounts for the fact that temperatures of chickens did not always correlate with temperatures of the holding facilities (II, V). A number of outlets did not reoffer for sale barbecued chickens held overnight; they discarded them or cut them up for sandwiches, salads, casseroles, etc.

Conclusions from surveys. The main conclusions drawn by the authors of these surveys are: (a) Survey I — barbecued chickens were often inadequately cooked to destroy pathogenic bacteria and were unhygienically retailed to the public. Stricter temperature controls were required, since those in existence at that time in Los Angeles County were ineffective. Storage of barbecued food at ≤ 50 or ≥ 140 F was recommended. Subsequently, regulations were enacted by the State of California requiring these temperatures for storing potentially hazardous food in Food Service Establishments (9).

(b) Survey II — 60% of holding facilities and 50% of barbecued chickens at the time of purchase were at temperatures which would permit growth of food-poisoning bacteria. It was recommended that barbecued food should be stored hot at ≥ 143 F.

(c) Survey III — in about 25% of the premises studied, temperatures of holding facilities were <140 F and poor hygienic practices were apparent.

(d) Survey IV — temperatures of holding facilities were <140 F in 53.3% of the premises studied. Deterioration in barbecue chicken operations in the Vancouver area, apparent from Survey III to Survey IV, resulted in a letter of guidance being sent to all provincial health units in British Columbia. Requirements included the cooking of poultry to 190 F, use of a meat thermometer, and storage of barbecued chickens at ≥ 140 F, or in the refrigerator.

(e) Survey V — this survey drew a distinction between freshly barbecued chickens and those that had been stored for some time. Freshly barbecued chickens seemed to be in a satisfactory condition, but 93% of barbecued chickens which had been refrigerated overnight and offered for sale on a subsequent day were displayed at temperatures considered unsafe, i.e., >40 or <143 F. Poor hygienic practices were apparent in many of the outlets. The authors recommended that hot-holding facilities should be maintained at 143 F or higher, should contain an easily-read thermometer, and should have a sign stating, "By law this facility must be maintained at 143 F or higher when it contains food".

(f) Survey VI — barbecued food seemed to be sold in a satisfactory manner, although a few of the smaller delicatessens tended to hold this food at temperatures <140 F for long periods of time.

(g) Survey VII — thermometers were lacking in cooking and holding facilities in all stores, and temperatures were unknown.

Assessment by Microbiological Analysis

Relatively little work has been done in analysing barbecued food for the presence of food-poisoning bacteria or their indicator organisms. In a trans-Canada survey Pivnick et al. (61) found that there was a 100-fold increase in standard plate count (SPC) in 55%, and a 10,000-fold increase in 15% of barbecued chickens incubated at 98.6 F for 8 hr compared with those analysed straight from the supermarket. Similarly, chickens were found to contain coagulase-positive staphylococci (22.8%) and presumptive *Clostridium perfringens* (8.3%) only after the incubation period. Pivnick et al. (60) also studied growth of food-poisoning bacteria artificially inoculated into freshly barbecued chickens (laboratory controlled); they found that *Staphylococcus aureus*, *C. perfringens*, and *Salmonella typhimurium* grew readily in chickens incubated at about 104 F and increased approximately 100,000 fold in 8 hr. Todd et al. (82) and Todd (*unpublished work*) analysed barbecued chickens bought in the Ottawa-Hull area of Canada. Freshly barbecued chickens contained relatively low numbers of bacteria (10-500 SPC/g); but counts in most stored chickens — those held overnight and offered for sale on a subsequent day — were higher (100-50,000 SPC/g). However, counts as high as 1.6×10^9 were obtained. There was presumptive evidence that many of the stored chickens contained organisms indicative of poor hygiene and faecal contamination; micrococci and staphylococci (79% of the chickens), faecal streptococci (39%), lactobacilli (24%), *C. perfringens* (16%), and *Escherichia coli* (10%).

Druilhet and Hensley (15) carried out a contract survey of Barbecue King rotisseries and holding facilities. Raw poultry and spare ribs, containing an average of 16,000 coliforms/g were barbecued at 300 F for 90 min. The food was found to be "sterile" after cooking, and when it was stored at 45 F or 140 F no bacteria were found until at least 24 hr. They also found that if barbecued poultry or spare ribs were cooled to 43 F, left overnight, and then reheated in the rotisserie the next morning, the food reached 140 F in 20 min and no bacteria were detected after this temperature was reached. If, however, the refrigerated barbecued poultry or spare ribs were rewarmed in the warming facilities set at 143 F, the products required 1 hr to reach 140 F and 3-4,000 bacteria/g were found.

These surveys show that freshly barbecued food contain virtually no bacteria, although spores may survive the heating process, but that storage of this food at temperatures considered unsafe (>45 F-

<140 F) may result in development of large numbers of bacteria. These bacteria, since they do not survive the cooking process, come from unsanitary handling practices after barbecuing and before storage. If stored at temperatures suitable for rapid growth, the contaminating bacteria could develop into many millions per gram in a short time. Reheating of refrigerated barbecued foods in the rotisserie is more satisfactory from a public health standpoint than rewarming in the holding facility, but reheating in the rotisserie is rarely practiced.

LEGISLATION

The extent of legislation that can be applied to barbecued food was found by contacting 116 different countries. Pertinent information including regulations was obtained from 64 of these countries, 54 states, 10 provinces, 6 territories, and 1 district. We did not consider municipal legislation, though it would be pertinent, because of the monumental task of collecting and collating it.

Control of the sale of barbecued food can be considered under 2 main categories: (a) regulations for food and (b) regulations for establishments (Table 7). The 2 categories of food are: (a) General Foodstuffs and (b) Potentially Hazardous Food; the latter includes milk products, meat, poultry, fish, and shellfish. The 3 categories of establishments are: (a) Food Service Establishments in which some form of service is implied, e.g., restaurants, cafeterias, or catering kitchens; (b) Retail Food Establishments in which the sale of food, but not its consumption on the premises, is implied, e.g., grocery stores, supermarkets or food markets; and (c) General Food Establishments in which the two other categories are included together with manufacturing and wholesale food premises.

To be legal, regulations concerning temperature have to be passed by a governing authority under an Act. Some authorities, however, issue letters of guidance or have certain policies which are recommended. Regulations and recommendations concerning temperatures for storing Potentially Hazardous Food are found in Tables 8, 9, and 10.

Barbecued food should be displayed or stored in a refrigerator or in a hot holding facility pending sale to prevent food poisoning. We consider that the refrigeration temperature should be ≤ 45 F for short term and ≤ 40 F for long term storage. However, it would be difficult to enforce a regulation which would require continual monitoring for removal of food from short to long term storage; in addition, one refrigerated temperature limit is easier for barbecue personnel and customers to remember. For this reason we prefer a refrigeration temperature of ≤ 40 F. The hot holding temperatures should be

TABLE 7. DEFINITIONS OF CATEGORIES IN FOOD REGULATIONS¹

| | | A. FOODS |
|----------------------------|---|--|
| General Foodstuffs | — | any food or drink, sold for human consumption, or any ingredient used in the preparation of food and drink. |
| Potentially Hazardous Food | — | any perishable food capable of supporting rapid and progressive growth of infectious or toxigenic organisms. |
| | | B. ESTABLISHMENTS |
| Food Service Establishment | — | any restaurant, industrial-feeding establishment, private, public or non-profit organization or institution serving food with or without charge. |
| Retail Food Establishment | — | any place which is used for the retail sale of food. |
| General Food Establishment | — | any place except a private home where food or food products are imported, prepared, processed, produced, transported, served, stored, sold or offered for sale for public and private consumption. General Food Establishment includes the two preceding categories. |

¹Our definitions based on an examination of regulations and other information from 135 countries, provinces and states.

TABLE 8. NUMBER OF PROVINCES AND TERRITORIES IN CANADA WITH REGULATIONS PERTAINING TO TEMPERATURES OF STORAGE OF POTENTIALLY HAZARDOUS FOODS^{1, 2, 3}

| Food Service Establishments | General Foods Establishments | General Foodstuffs | Barbecued foods | Temperature regulations (°F) | | Adequacy of regulations |
|-----------------------------|------------------------------|--------------------|-----------------|------------------------------|------|-----------------------------|
| | | | | Low | High | |
| 1 | 1 | 1 | 0 | ≤40 | ≥150 | Considered to be safe |
| 0 | 0 | 0 | 1 ⁴ | ≤40 | >150 | Considered to be safe |
| 1 | 0 | 0 | 0 | <40 | >140 | Considered to be safe |
| 0 | 0 | 0 | 1 ⁴ | Refrigerated | ≥143 | Requires more definition |
| 0 | 0 | 0 | 1 ⁴ | Refrigerated | ≥140 | Requires more definition |
| 1 | 0 | 0 | 0 | ≤40 | None | Considered to be inadequate |
| 1 | 1 | 0 | 0 | ≤45 | None | Considered to be inadequate |
| 4 | 0 | 3 | 0 | ≤50 | None | Considered to be inadequate |

¹Provinces may have regulations for more than one kind of establishment or foodstuff.

²Ten provinces and 2 territories; one province has no regulations.

³Information received up till December 1971.

⁴Recommendations issued by provincial governments; these are not regulations.

TABLE 9. NUMBER OF STATES IN THE UNITED STATES OF AMERICA WITH REGULATIONS PERTAINING TO TEMPERATURES OF STORAGE OF POTENTIALLY HAZARDOUS FOODS^{1, 2, 3}

| Food Service Establishments as defined in Table 7 | as defined in Table 7 plus grocery stores | Retail Food Establishments | General Food Establishments | General Foodstuffs | Cooked meats including barbecued food | Temperature regulations (°F) | | Adequacy of regulations |
|---|---|----------------------------|-----------------------------|--------------------|---------------------------------------|------------------------------|------|-----------------------------|
| | | | | | | Low | High | |
| 2 | 1 | 0 | 0 | 0 | 0 | ≤40 | ≥150 | Considered to be safe |
| 0 | 0 | 0 | 0 | 0 | 1 | ≤40 | ≥143 | Considered to be safe |
| 21 | 5 | 4 | 7 | 1, 1 ⁴ | 2 ⁴ | ≤45 | ≥140 | Considered to be safe |
| 0 | 0 | 0 | 1 | 0 | 0 | <45 | >140 | Considered to be safe |
| 0 | 0 | 0 | 1 ⁴ | 0 | 0 | ≤45 | ≥149 | Considered to be safe |
| 1 | 0 | 1 | 1 | 0 | 1 ⁴ | ≤45 | ≥150 | Considered to be safe |
| 2 | 0 | 1 | 0 | 0 | 2 ⁴ | ≤50 | ≥140 | Considered to be inadequate |
| 1 ⁴ | 1 | 0 | 0 | 0 | 0 | ≤50 | ≥143 | Considered to be inadequate |
| 0 | 0 | 0 | 0 | 0 | 1 ⁴ | None | ≥140 | Considered to be inadequate |
| 0 | 0 | 0 | 0 | 0 | 2 ⁴ | None | ≥150 | Considered to be inadequate |
| 0 | 0 | 1 | 0 | 0 | 0 | ≤40 | None | Considered to be inadequate |
| 1 | 0 | 1 | 2 | 0 | 0 | ≤45 | None | Considered to be inadequate |
| 3 | 0 | 3 | 3 | 0 | 0 | ≤50 | None | Considered to be inadequate |

¹Includes 49 states, 2 territories and 1 federal district (in text all considered states); 2 states and the territories have no regulations.

²States may have regulations for more than one kind of establishment.

³Information received up till May 1971.

⁴Recommendations issued by state departments of health or agriculture; these are not regulations.

TABLE 10. OTHER COUNTRIES WITH REGULATIONS PERTAINING TO TEMPERATURES OF STORAGE OF POTENTIALLY HAZARDOUS FOODS^{1, 2}

| Food Service Establishments | General Food Establishments | General Foodstuffs | Potentially Hazardous Foods including barbecued foods and other ready-to-eat products | Temperature regulations (°F) | | Reference | Adequacy of regulations |
|-----------------------------|-----------------------------|--------------------|---|------------------------------|------|-----------|-----------------------------|
| | | | | Low | High | | |
| | | | Denmark ³ | <41 | ≥158 | (73) | Considered to be safe |
| | | | Finland ³ | ≤41 | ≥158 | (81) | Considered to be safe |
| | | | Czechoslovakia | ≤39.2 | >149 | (17) | Considered to be safe |
| Denmark ³ | | | | <41 | >149 | (73) | Considered to be safe |
| | | | Norway ³ | ≤39.2 | ≥140 | (27) | Considered to be safe |
| | | | Bulgaria | ≤39.2 | ≥140 | (19) | Considered to be safe |
| | | | Sweden | ≤42.8 | ≥140 | (11) | Considered to be safe |
| | Scotland | | | Refrigerated | >145 | (22) | Requires more definition |
| | England, Wales | | | <50 | ≥145 | (21) | Considered to be inadequate |
| | | | Hong Kong ³ | ≤50 | ≥145 | (80) | Considered to be inadequate |
| | | | Poland | 35.6-46.4 | None | (62) | Considered to be inadequate |
| | | | Hungary | ≤39.2 | None | (54) | Considered to be inadequate |
| | | | Ireland ³ | <40 | None | (90) | Considered to be inadequate |
| Mauritius | | | | ≤40 | None | (50) | Considered to be inadequate |
| | | | Switzerland ³ | ≤41 | None | (33) | Considered to be inadequate |
| | | | USSR | ≤46.4 | None | (53) | Considered to be inadequate |
| | | Columbia | | Refrigerated | None | (20) | Considered to be inadequate |
| | | Spain | | Refrigerated | None | (74) | Considered to be inadequate |

¹Total of 64 countries; 46 of these countries have no regulations.

²Information received up till May 1971.

³Recommendations issued by government departments; these are not regulations

≥140 F for safe storage. We consider as adequate those regulations or recommendations that specify ≤45 F or ≥140 F; we consider as unsafe storage between these limits.

In the following sections legislation governing barbecued food is presented and discussed. Canada and the United States of America are treated separately from other countries because of their large sizes, the diversity of regulations within the countries and the relatively large volume of pertinent information available to us.

Canada

There are no federal regulations or guide lines concerning temperature control in the preparation, storage, and sale of food in Canada. It is not surprising, therefore, that there is considerable variation in temperature regulations in the 12 provinces and territories; only a few of these have adequate regulations (Table 8).

Although there have been only three reported food-poisoning outbreaks in Canada (6, 49, 59), confidential information indicates that there have been more. As the retail sale of barbecued food appears to be increasing, additional outbreaks are likely to occur unless suitable temperature controls are incorporated into provincial and territorial regulations, and enforced. Guidance from the federal government to obtain more uniform and safe regulations appears desirable.

United States of America

Food Service Establishments receive adequate

guidance for establishing regulations for the safe handling of food from the US Department of Health, Education, and Welfare (USDHEW). The USDHEW Food Service Sanitation Manual (23) recommends temperature limits for Food Service Establishments of ≤45 and ≥140 F. The National Sanitation Foundation, a non-official and non-commercial agency, recommends in Standard No. 7 (76) and No. 4 (75), respectively, that equipment should hold cold food at ≤40 F for long term storage and ≤45 F for short term storage, and hot food at ≥140 F.

The influence of USDHEW recommendations to Food Service Establishments is obvious; 28 of the states have regulations of ≤45 and ≥140 F. In addition, 4 states have more stringent requirements. Thus, 32 states have adequate regulations concerning temperature for the holding of food in Food Service Establishments (Table 9). Data for Retail Food Establishments and General Food Establishments are also presented in Table 9.

Retail Food Establishments are not uniformly regulated, possibly because of lack of federal guidelines. Ten states have regulations under Retail Food Establishments, and another 9 states have regulations for grocery stores incorporated with regulations for Food Service Establishments; 12 of these 19 states have regulations considered to be safe.

General Food Establishments, which includes both Food Service Establishments and Retail Food Establishments, is a category of establishment not usually considered in state regulations. Only 11 of the states have regulations for General Food Establish-

ments; of these, 7 have adequate regulations.

Barbecued foods are not governed by specific regulations, but 5 states have recommendations; 3 of these appear adequate.

In the United States there are many problems in the interpretation of areas of responsibility for handling of foods. The State Departments of Health are usually responsible for Food Service Establishments, and the State Departments of Agriculture for Retail Food Establishments. However, the regulations governing these two kinds of establishments are not usually complementary. The procedure for handling Potentially Hazardous Food, as described by the USDHEW, could be profitably adopted by all regulatory agencies.

Other countries

Some countries have regulations pertaining to temperatures for storing food, but most do not. From the information that we received, 18 countries have such regulations; 46 countries do not (Table 10).

Food Service Establishments is a category not usually regulated in the 18 countries. General Food Establishments and General Foodstuffs are more frequently regulated, but most of the 18 countries have regulations or specific recommendations for the category Potentially Hazardous Food; this category would include barbecued food.

Only 6 of the 18 countries have adequate regulations or recommendations for safe temperatures of storage; these are all from Europe — Bulgaria, Czechoslovakia, Denmark, Finland, Norway, and Sweden. These temperatures differ little from those considered safe in Canada and the USA. The remaining 12 countries have indefinite, inadequate, or non-existent low or high temperature limits (Table 10). In addition to the information tabulated in Table 10, maximum times (3-72 hr) for storage of specific cooked foods are found in the regulations of Bulgaria, Czechoslovakia, Hungary, Poland, and the USSR. Also, Scotland requires that cooked food be reheated to >180 F before serving.

Several countries are at present formulating new or improved regulations regarding temperature control of food. Four countries and 1 state (in Australia) are proposing adequate regulations; 2 countries are proposing regulations that we consider unsafe.

CONCLUSIONS

There is an increasing demand for barbecued food in many countries of the world and, in many of these, conditions for storing the food pending sale are conducive to contamination by food-poisoning bacteria and their subsequent growth. Food-poisoning outbreaks have occurred and will continue to do so unless adequate regulations are promulgated and en-

forced. Public health authorities in several countries with regulations admit that there are difficulties in enforcement. Some of the problems of enforcement in Ontario, Canada, have been discussed (82).

Regulations concerning temperature for holding barbecued food must include maximum refrigerated and minimum hot temperatures. The refrigerated temperature can be ≤ 45 F for short term storage and ≤ 40 F for long term storage, although we prefer one refrigerated temperature limit — ≤ 40 F — irrespective of storage time for convenience and ease of enforcement; the hot temperature should be ≥ 140 F. Our reasons for recommending ≥ 140 F are: (a) commercial warm holding units may fluctuate ± 10 F (E. Todd unpublished work); (b) the D_{140} (minutes to destroy 90% of vegetative cells) of most foodborne pathogens is about 10 min or less; and (c) unsold meats refrigerated overnight are returned to the warming cabinet usually without reheating; the potential hazard of this practice has been documented by Senn and Williams (72).

Temperatures of 150 F or greater for hot storage, although undoubtedly safe, are not necessary. They may even be detrimental to acceptance of a minimum high temperature because many vendors of barbecued meats consider that the organoleptic quality of the food is destroyed by storage at such temperatures. This is indicated by current methods of vending (34). We do not insist that temperature control of barbecued food is the only method of maintaining a safe and wholesome product, but we consider it the most effective way.

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THE FATE OF *SALMONELLA TYPHIMURIUM* AND *STAPHYLOCOCCUS AUREUS* IN COTTAGE CHEESE WHEY¹

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ABSTRACT

Cottage cheese whey was inoculated with *Salmonella typhimurium* and *Staphylococcus aureus* and stored for up to 9 days at 5, 25, and 35 C. Salmonellae could not be isolated from samples stored longer than 3 days when the inoculum provided 1.9×10^3 cells per milliliter or less. Staphylococci were more resistant to the adverse effect of pH than salmonellae. When the initial population was 1.0×10^6 cells per milliliter, salmonellae counts decreased 99.99% in one day at both 25 and 35 C. Under similar conditions, staphylococcal counts decreased about 90% at 25 C and 99% at 35 C during the first day of storage. Storage at 5 C had a very limited effect on the viability of staphylococci. The rapid inactivation of both pathogens at 25 C and 35 C could be explained primarily by the low pH (4.5 - 4.6) resulting from the presence of lactic acid.

There is interest in the food industry to use fresh liquid cottage cheese whey in frozen desserts, water ices, fruit flavored drinks, and other products (2, 12). Methods have been developed to dry cottage cheese whey and the dried product is gaining acceptance by the food processing industry. Cooking temperatures used to manufacture cottage cheese are usually sufficient to eliminate salmonellae (8), staphylococci (9), and various spoilage organisms (4) that might be present. A recent study in this laboratory (15) has shown freshly drawn cottage cheese whey to be of excellent microbial quality. Greater use of cottage cheese whey as a food ingredient, however, will result in more handling, storage, and transportation of whey. From a public health aspect, the possible transmission of pathogenic organisms from contaminated whey to other food products has not been clarified. The potential of cottage cheese whey as a public health hazard has been implied by Helm and Wedermann (7). They reported the survival of *Salmonella paratyphi* B and *Salmonella enteritidis* in acid and rennet wheys. Prost and Riemann (14) suggested that a pH value below 4.5 is bactericidal for salmonellae. However, Chung and Goepfert (3) reported that salmonellae were able to initiate growth in tryptone-yeast extract-glucose broth acidified with lactic acid to pH 4.4. Goepfert et al. (6) also reported that a pH value of 4.9 (lactic acid) had little or no

detrimental effect on multiplication of *Salmonella typhimurium* in skim milk. The inhibitory effect of a low pH value or a relatively acidic food on salmonellae is influenced by storage temperature (6, 13) and the particular acid present (5). The relationship between acidity and the growth of *Staphylococcus aureus* in milk has been examined by Minor and Marth (10). The threshold for inhibition of growth by lactic acid was reported to be pH 5.1 - 5.2.

Even in the absence of growth, addition of contaminated whey to other foods might establish conditions that are favorable for bacterial growth and/or survival. This study was initiated to ascertain the fate of *S. typhimurium* and *S. aureus* when inoculated into fresh liquid cottage cheese whey and stored at several temperatures.

MATERIALS AND METHODS

Whey samples

Cottage cheese whey was obtained from a local manufacturer during the normal draining of vats. Samples were transported to the laboratory in sterile cans and refrigerated. All experiments were initiated within 18 hr from the time of sampling.

In later experiments, whey was sterilized by centrifugation at $6,000 \times g$ for 30 min followed by filtration through a $0.45\text{-}\mu$ membrane (Millipore Corp.). Neutral whey was obtained by adjusting the pH to 6.8 with 40% sodium hydroxide. Noncultured or directly acidified whey was prepared by adding lactic acid to HTST pasteurized skimmilk until pH 4.6 was reached. The acidified milk was then "cooked" at 130 F for 15 min and filtered through sterile cheese cloth into sterile flasks. The resulting filtrate was cooled and designated "directly acidified whey."

Media and cultures

All the bacteriological media used in this study were from BBL, Division of Bioquest. The cultures were *Salmonella typhimurium* ATCC 11331 and *Staphylococcus aureus* ATCC 8095.

Inoculation procedure

Cultures were grown on nutrient agar slants with incubation at 35 C, and then were stored at 5 C. One hundred milliliters of trypticase soy broth (TSB) were inoculated and incubated at 35 C for 18 hr. A subculture (10% inoculum) was made into TSB followed by incubation at 35 C for 3 hr. Cells were harvested by centrifugation at $6,000 \times g$ for 20 min at 2 C and resuspended in 10 ml of 0.05 M sodium phosphate buffer, pH 6.8. Dilutions were prepared to obtain the desired cell concentrations and the whey was inoculated. Aliquots were immediately removed for the 0 hr

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TABLE 1. EFFECT OF STORAGE TEMPERATURE AND LEVEL OF CONTAMINATION ON THE SURVIVAL OF *Salmonella Typhimurium* IN WHEY

| Level of contamination salmonellae/ml | Confirmed salmonellae surviving ¹ | | | | | | | | | |
|---------------------------------------|--|-----|-----|-----|------|-----|-----|------|-----|---|
| | Days: | 5 C | | | 25 C | | | 35 C | | |
| | | 3 | 6 | 9 | 3 | 6 | 9 | 3 | 6 | 9 |
| 1.1×10^6 | ++ ³ | ++ | --- | -+ | --- | --- | -+ | --- | --- | |
| 1.2×10^5 | +— | --- | --- | --- | --- | --- | --- | --- | --- | |
| 1.8×10^4 | +— | --- | --- | --- | --- | --- | --- | --- | --- | |
| 1.5×10^3 | --- | --- | --- | --- | --- | --- | --- | --- | --- | |
| 4.1×10^2 | --- | --- | --- | --- | --- | --- | --- | --- | --- | |
| 1.2×10^1 | --- | --- | --- | --- | --- | --- | --- | --- | --- | |

¹Positive growth in selenite cystine broth, followed by streaking on brilliant green agar and picking colonies to triple sugar iron agar and confirmation by a slide agglutination with polyvalent "0" antiserum.

²Numbers determined by pour plates on trypticase soy agar immediately following inoculation of the whey.

³Positive for salmonellae (+), none detectable (-), each symbol represents one experiment.

TABLE 2. EFFECT OF STORAGE TEMPERATURE AND LEVEL OF CONTAMINATION ON THE SURVIVAL OF *Staphylococcus Aureus* IN WHEY

| Level of contamination staphylococci/ml | Confirmed staphylococci surviving ¹ | | | | | | | | | |
|---|--|-----|-----|-----|------|-----|-----|------|-----|-----|
| | Days: | 5 C | | | 25 C | | | 35 C | | |
| | | 3 | 6 | 9 | 3 | 6 | 9 | 3 | 6 | 9 |
| 1.2×10^6 | +++ ³ | ++ | ++ | ++ | ++ | ++ | +— | --- | --- | --- |
| 1.4×10^5 | ++ | ++ | ++ | ++ | ++ | +— | --- | --- | --- | |
| 1.1×10^4 | ++ | ++ | ++ | ++ | --- | --- | --- | --- | --- | |
| 3.2×10^3 | ++ | ++ | -+ | ++ | --- | --- | --- | --- | --- | |
| 2.1×10^2 | ++ | ++ | -+ | --- | --- | --- | --- | --- | --- | |
| 1.9×10^1 | ++ | ++ | --- | --- | --- | --- | --- | --- | --- | |

¹Typical staphylococcal colonies were picked from Vogel-Johnson agar and confirmed by gram-staining and a coagulase tube test using rabbit plasma.

²Numbers determined by pour plates on Brain Heart Infusion Agar immediately following inoculation of the whey.

³Positive for staphylococci (+), no detectable staphylococci (-), each symbol represents one experiment.

values and the remainder of the samples were incubated at 5, 25, and 35 C in thermostatically controlled water baths.

Detection of salmonellae

The recommended sample volume to be used in the isolation of salmonellae varies (1, 11, 18). The method used in this study was similar to that outlined by the American Public Health Association (1). Twenty milliliters of whey were neutralized with a predetermined volume of sterile 40% sodium hydroxide and added to 80 ml of selenite cystine broth (SCB) followed by incubation at 35 C for 24 hr. Loopsful of the SCB were then streaked on the surface of brilliant green agar (BGA) plates and incubated at 35 C for 48 hr. Typical *Salmonella* colonies were picked, and stab and streak inoculations were made into triple-sugar-iron agar (TSI) slants followed by incubation at 35 C for 18 hr. Tubes showing *Salmonella* reactions were confirmed by a slide agglutination with polyvalent "0" antiserum (BBL).

Enumeration of salmonellae

Viable numbers of salmonellae were determined by streaking in triplicate 0.1 ml of appropriate dilutions on the surface of BGA plates followed by incubation at 35 C for 48 hr. Routinely, typical salmonellae colonies were confirmed by their TSI reaction and by slide agglutination.

Detection of staphylococci

The presence of staphylococci was determined by spreading (0.5 ml) of the samples on the Vogel-Johnson agar (VJ) followed by incubation at 35 C. Plates were examined following 24 and 48 hr of incubation. Typical staphylococcal colonies were picked for confirmation by gram staining and a coagulase tube test using rabbit plasma (BBL).

Enumeration of staphylococci

Viable numbers of staphylococci were determined by streaking in triplicate 0.1 ml of appropriate dilutions on the surface of mannitol salt agar (MSA) plates, followed by incubation at 35 C for 36 hr. Typical staphylococcal colonies were routinely confirmed by gram staining and the coagulase test.

Non-selective enumeration

Whey samples were also pour-plated with either TSA or brain heart infusion agar (BHIA) followed by incubation at 35 C for 24 hr.

RESULTS AND DISCUSSIONS

The initial pH values of whey samples ranged from 4.5 to 4.6. During storage for up to 6 days, pH did not vary more than 0.1 unit. Microbiological analysis revealed that neither salmonellae nor staphylococci could be isolated from any of the fresh whey samples or from any of the uninoculated controls following storage.

To simulate the possible contamination of whey, samples were inoculated with various initial populations of salmonellae or staphylococci and stored for up to 9 days. Many of the inocula used represented levels of contamination that would never be encountered in practice. Salmonellae could not be recovered from whey following 3 days of storage when initial

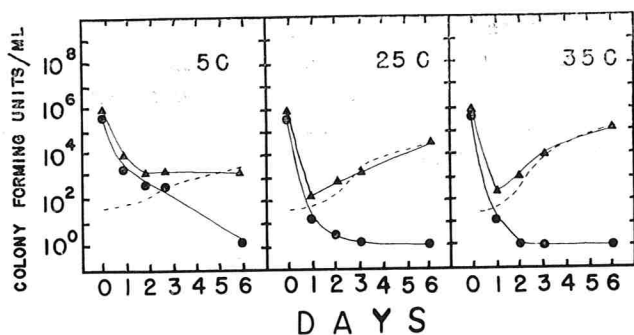


Figure 1. Survival curves for *Salmonella typhimurium* in whey when enumerated on brilliant green agar (BGA) (closed triangles) and trypticase soy agar (TSA) (closed circles). Dash line is a non-inoculated control enumerated on TSA.

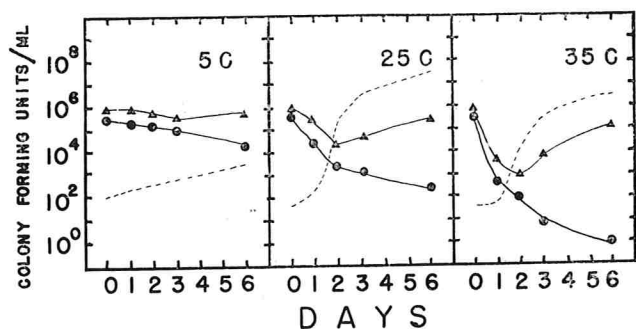


Figure 2. Survival curves for *Staphylococcus aureus* in whey when enumerated on mannitol salts agar (MSA) (closed triangles) and brain heart infusion agar (BHIA) (closed circles). Dash line is a non-inoculated control enumerated on BHIA.

populations were 1.5×10^3 /ml or less (Table 1). A comparison of data in Tables 1 and 2 indicates that *S. aureus* withstood the adverse effect of low pH more readily than *S. typhimurium*. Storage at 5 C had a very limited effect on the recoverability of staphylococci (Table 2). Even low levels of staphylococci (19/ml) persisted for 6 days at 5 C (Table 2). Storage temperature was a significant factor in the survival of both pathogens in whey. Storage at 5 C was considerably less detrimental than storage at either 25 or 35 C. This suggests that there would be very little advantage to refrigerating whey during storage; however, the effect of storage temperature on the growth of the normal flora of cottage cheese whey needs to be considered.

The rate of inactivation of both cultures was further examined by inoculating whey with approximately 1.0×10^6 viable cells per milliliter and enumerating survivors during 6 days of storage at 5, 25, and 35 C (Fig. 1 and 2). *S. typhimurium* rapidly lost its ability to form colonies on BGA and TSA. For example, storage at 25 or 35 C resulted in at least 99.99% inactivation after 1 day (Fig. 1). Storage of salmonellae for 1 day at 5 C result in at least 99.0% inactivation. Loss of viability by *S. aureus*

was not as rapid, although viable cell numbers did decrease significantly during storage, particularly at 25 and 35 C (Fig. 2).

Counts on the nonselective plating media (TSA and BHIA) were always higher than counts on BGA and MSA (Fig. 1 and 2). The selective properties of the media and the possible presence of injured cells resulting from the stress of low pH could account for the difference. However, the increase in the population of bacteria other than salmonellae and staphylococci during the later days of storage is also reflected in the counts on TSA and BHIA. The dotted lines in both Fig. 1 and 2 represent the increase in the normal flora of whey during storage. Higher populations were recorded when plating was done with BHIA as compared to TSA. It is also noteworthy (Fig. 2) that uninoculated whey (controls) had higher counts than the same whey when inoculated with *S. aureus*. This inhibition of the natural flora by the pathogen was not observed in experiments with salmonellae (Fig. 1). The fastidious requirements of *S. aureus* and the resulting competition for nutrients might explain this observation.

The inhibitory effect of various acids, particularly

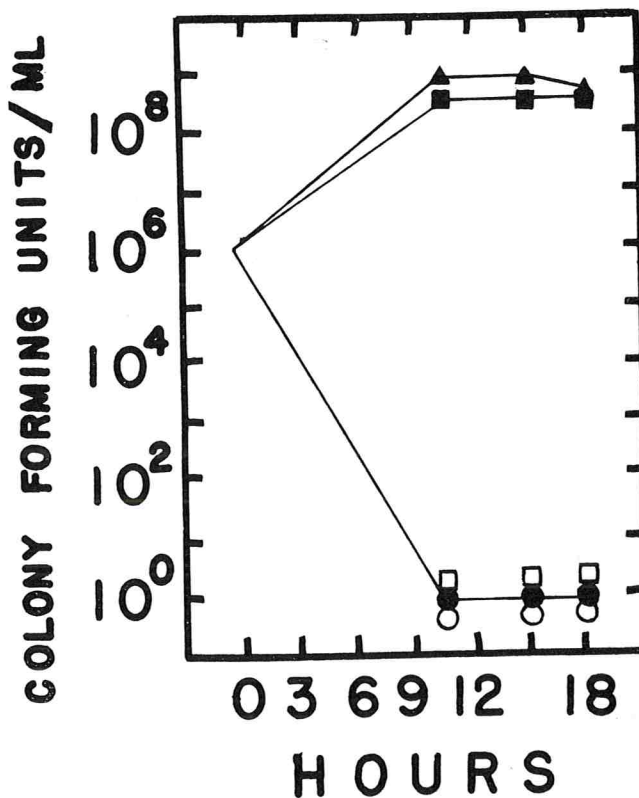


Figure 3. Fate of *Salmonella typhimurium* in whey (open circles), neutralized whey (closed triangles), filtered sterilized whey (closed circles), directly-acidified neutralized whey (closed squares) and directly acidified whey (open squares) when stored at 35 C and enumerated on brilliant green agar (BGA).

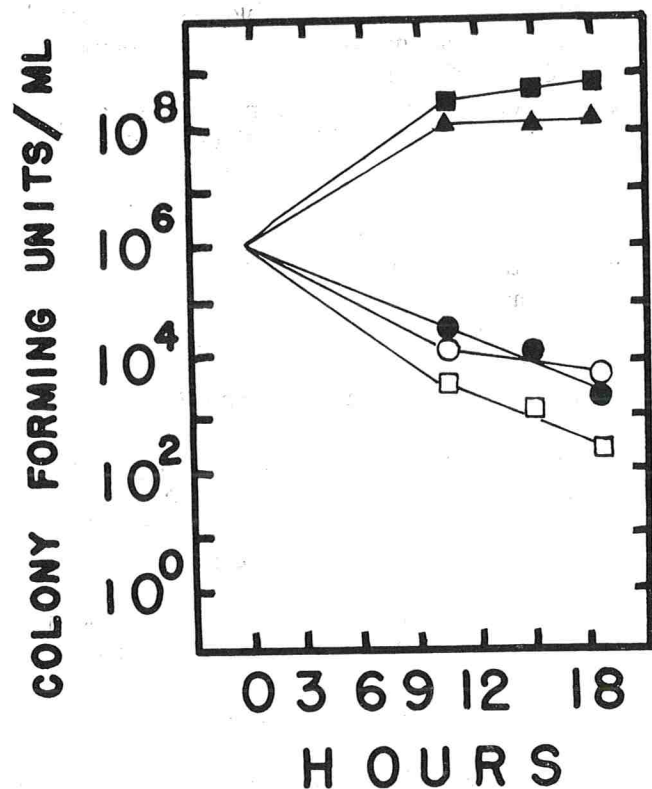


Figure 4. Fate of *Staphylococcus aureus* in whey (open circles), neutralized whey (closed triangles), filtered sterilized whey (closed circles), directly-acidified neutralized whey (closed squares), and directly acidified whey (open squares) when stored at 35 C and enumerated on mannitol salts agar (MSA).

lactic acid, on salmonellae and staphylococci has been shown (10, 17). Loss of viability by *S. typhimurium* and *S. aureus* in this experiment, therefore, might be explained by the presence of lactic acid. In addition, it is also known that many lactic acid bacteria can inhibit various pathogens (16). The presence of an inhibitory substance in the cottage cheese whey resulting from the starter culture is possible. Differences between growth of the pathogen in neutralized whey compared to directly-acidified neutralized whey (no starter culture activity) were variable (Fig. 3 and 4) and the possible presence of an inhibitor produced by the starter culture can not be completely eliminated. Presence of other organisms in whey might also be responsible for some culture interactions, particularly at 25 and 35 C where an increase in non-pathogenic bacteria occurred. To eliminate the possibility of culture interactions during storage, whey was filter-sterilized to remove the normal flora and then was inoculated. Rapid inactivation of both pathogens was observed (Fig. 3 and 4), suggesting that the increase in the normal flora was not responsible for death of salmonellae and staphylococci. As is shown by data in Fig. 3 and 4, fresh cottage cheese whey contains

all the necessary nutrients for abundant growth of salmonellae and staphylococci when the pH is favorable. It is suggested that the major factor responsible for loss of viability by both pathogens was the unfavorable pH caused by lactic acid.

ACKNOWLEDGMENT

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ECOLOGICAL SIGNIFICANCE OF THE DISCHARGE OF TREATED WASTEWATERS INTO COASTAL WATERS¹

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ABSTRACT

The Southern California Coastal Water Research Project (SCCWRP) is attempting to attain a substantial understanding of the ecology of the coastal waters of Southern California. The results are expected to provide insight into the past, present, and predicted effects of man on the ecology, particularly those caused by wastewater discharges. The findings should be useful in efforts to limit harmful effects and to promote enhancement of the coastal environment. The major effort thus far has been an information search in 17 task areas of physical and chemical oceanography, marine biology, and environmental engineering. In addition, several new research projects have been started under SCCWRP direction.

This paper discusses some of the technical problems associated with such an effort such as quantifying the natural fluctuations of physical, chemical, and biological parameters; establishing environmental criteria; and correlating observed effects with pollutant distributions.

The Southern California region is especially significant with respect to the problem of water pollution control because it exemplifies the impact of a highly developed coastal society on the environment and may serve as a model for the future. It is also subject to natural periodic and aperiodic fluctuations of environmental conditions which range from days to decades. The SCCWRP, a relatively new, goal-oriented research effort, has been established to study the coastal water environment especially with respect to the effects of wastewater discharges. The project may draw upon both the expertise and facilities of the academic and industrial communities as well as benefit from the experience and technology of local sanitation agencies. The project was started and initially sponsored by the five local government agencies responsible for wastewater disposal in the region - Ventura County, the Cities of San Diego and Los Angeles, and County Sanitation Districts in Orange County and Los Angeles County. A Commission, the SCCWRP Authority, was established by the sponsors to assume control of the project and to be responsible to the public. The principal contributors to the project effort are shown in Table 1.

The goals and objectives of the project are to: (a) determine the input rates and distribution of the substantial and trace-level organic and inorganic ma-

terials entering the coastal waters; (b) investigate the natural phenomena of the Southern California Bight in present and recent times; (c) determine the effects of man on the coastal waters and distinguish them from natural phenomena; (d) develop indices of environmental health; (e) develop the capacity to predict man-induced effects on the coastal waters; and (f) determine the methods by which the coastal water environment may be enhanced.

THE SOUTHERN CALIFORNIA BIGHT

The Southern California coastal region forms the northern half of the Southern California Bight, a complex feature of the Pacific shoreline of North America. The Bight is defined by the coastal indentation that stretches from Point Conception southward approximately 300 miles to Cape Colnett in Baja California, Mexico (Fig. 1). Although the area displays an unusual bathymetry or bottom topography consisting of a series of deep coastal basins, troughs, and submarine canyons, the Bight is much more than just a geological feature. Point Conception constitutes a boundary to a large number of organisms, marking the northern limits of many creatures common to the south and similarly limiting many of more northern habitat. Further, the Bight acts as a breeding and nursery grounds for the larval and juvenile stages of species with widespread adult forms. Thus, the region is potentially sensitive to the effects of pollution by virtue of the greater sensitivity of youthful forms and because of the long residence-time of waters in the Bight.

Recirculation and retention of substances within the region involve processes of some complexity. As an example, although substantial primary productivity of new food material takes place in the Bight, the creatures of the region are major consumers of food material that has been elaborated in the more northerly portions of the California Current System. Thus major Southern California creatures may be oriented somewhat higher in the trophic system than those of more northern latitudes (i.e., more food web steps intervene between them and the primitive plant material). As a result, certain chemical substances, including pollutants, entering the California Current to the north, that is from San Francisco Bay, the Columbia River, Puget Sound, or by atmospheric

¹Presented at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, San Diego, California, August 15-19, 1971.

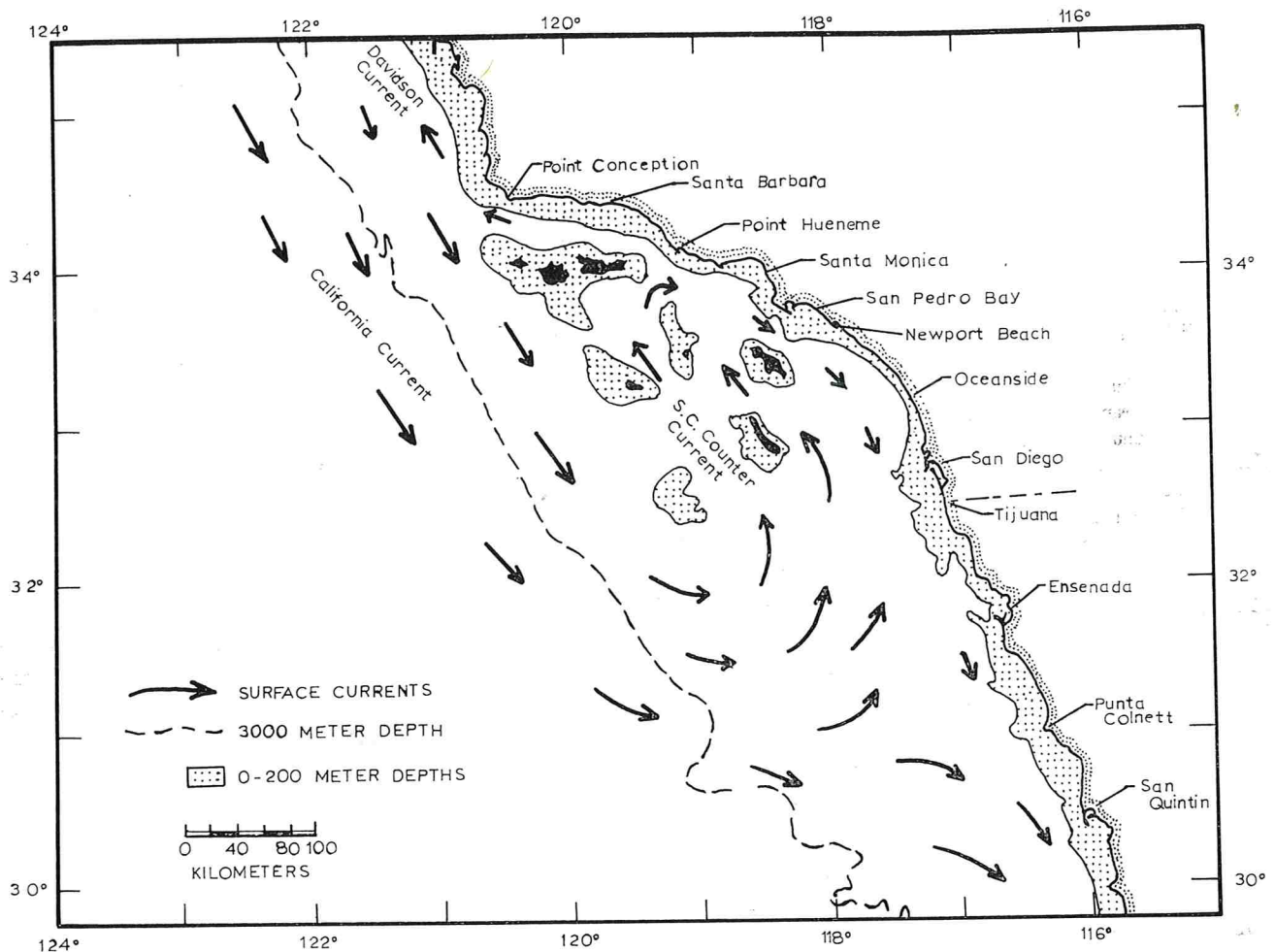


Figure 1. Estimated average surface circulation in the Southern California Bight Region

TABLE I. ORGANIZATION CHART FOR THE SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT AUTHORITY

COMMISSION MEMBERS

Bert Bond, President
Lindsay Parsons, Vice President
Helen Cobb
Thomas E. Laubacher
L. E. Timberlake

ALTERNATES

T. D'Arcy Quinn
Clifton C. Miller
Bob Martinet
Franklin R. Jewett
William S. Peterson

CONSULTING BOARD

John D. Isaacs, Chairman
Richard K. C. Lee
Erman A. Pearson
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PROJECT MANAGER

George E. Hlavka, Ph.D.

PROJECT ADMINISTRATIVE OFFICER

Charles N. Halgren

SECRETARY

Elli Steiger

SENIOR ENVIRONMENTAL SPECIALISTS

Irwin Haydock, Ph.D.
James Jones, Ph.D.
Alan J. Mearns, Ph.D.
Chen-Shyong Young
David R. Young, Ph.D.

OTHER SPECIALISTS

Tareah J. Hendricks, Ph.D.
Marjorie J. Sherwood

ADMINISTRATIVE ASSISTANT

Ginny Barney

TECHNICIANS

Joseph Johnson
Thomas S. Sarason
Kimm W. Crawford
Deirdre J. McDermott
Gerald I. Shiller
Marcia Kerwit
Ricard G. Gammon

fallout in the West Wind Drift, have been subjected to more concentrating steps than those similar substances entering the system locally. The parent waters of the Southern California Bight therefore provide important inputs to the area and, for some

materials, perhaps dominate other sources in importance.

In addition, the location of the Bight at the point of convergence of northern, western, southern, and upwelled waters results in natural fluctuations of

conditions that limit the predictive value of provincial studies.

RESEARCH NEEDS FOR THE SOUTHERN CALIFORNIA COASTAL WATERS

The Southern California region sustains a highly developed society, comprising 5.5% of the United States population, whose ebullience is reflected in the demands made upon the marine resources for food, recreation, transportation, oil and power production, and waste disposal. In order to achieve a continuing satisfactory balance between these conflicting uses of the environment, the health of the region must be maintained. In an excellent 1970 report by the National Academy of Sciences (NAS) and the National Academy of Engineering (NAE) (1) the minimum effort required for a coastal zone study involving physical processes, chemical factors, biological effects, and monitoring programs was estimated to be 2660 man-years. This compares with about 5 man-years for SCCWRP to date! Thus, SCCWRP can only do a fraction of the work that needs to be done, and the necessity for evaluating and selecting programs most significant for the Southern California region must be stressed. The initial SCCWRP effort therefore involved an information search in some seventeen task areas of physical and chemical oceanography, marine biology, public health, and environmental engineering. This search is a continuing effort stimulated by previous investigations and by on-going research activities. The active research phase of the SCCWRP program, though still in the planning stages, is being developed from information collected and evaluated during Phase 1. Several studies, already initiated, were also emphasized as high-priority items in the NAS-NAE report. These involve a Bight inventory of waste inputs and distributions and an attempt to establish quantitative biological indices of waste effects.

INVENTORY

As part of the chemical inventory of inputs and distributions, three programs are currently underway to assess and quantify heavy metals.

Chemical analysis of sediments

SCCWRP was able to involve the excellent laboratory of Dr. James Arnold, University of California, San Diego, in the analysis of sediment samples from the vicinity of the major Southern California wastewater outfalls. In cooperation with Mr. James Gallo-way, SCCWRP enlarged an existing program with respect to both the number of metals measured (from 4 to 10) and the number of locations involved. Sediment samples taken from the vicinity of the Hyper-

ion, Whites Point, Point Loma, and old and new Orange County outfalls have been analyzed for chromium, copper, iron, manganese, silver, and zinc. Horizontal concentration contours based on several thousand measurements have been plotted. Preliminary results indicate that surface sediments in close proximity to several of the major sewage outfalls display metal concentrations significantly altered with respect to concentrations in deeper and in more distant surface sediments. These values provide information about the distributions of these metals following their introduction into the coastal waters and demonstrate the geographical scope of influence exerted by the discharges on the sediments. Findings should be published this year and should prove useful in defining the geographical limits of monitoring programs.

In conjunction with the SCCWRP effort to measure the heavy metals in the sediments of the Bight, use has been made of cores or vertical samples which were collected by Dr. Andrew Soutar of Scripps Institution of Oceanography from the floor of the anaerobic Santa Barbara Basin. These sediments provide an undisturbed chronological record of oceanographic and biological events occurring in the area. In most areas the larger bottom burrowing animals destroy the chronology of the record, but in the Santa Barbara Basin, the continued lack of oxygen excludes the presence of these organisms. Depth profiles for cadmium, lead, and the metals listed above have been prepared from a varved core covering the last 130 years. Analyses were made at five-year intervals. Results are currently being evaluated and may provide, for the first time, an accurate and continuous historical record of the heavy metal inputs to the coastal waters from both natural and man-induced events.

Chemical analysis of organisms

In conjunction with the continuing SCCWRP program to measure the levels of important constituents in the various reservoirs of the Bight, trace heavy metals, chlorinated hydrocarbons, and radio-nuclides are being investigated in representative and ubiquitous organisms collected along five lines radiating from the heavily populated Los Angeles area. Results should illustrate the presence and magnitude of any concentration gradients. Specimens of *Mytilus californianus*, *Pollicipes polymerus*, *Pisaster ochraceus*, and *Haliotis cracherodii* have already been collected from up to eleven coastal stations and six island stations. Selected tissues from these specimens are presently being analyzed via emission spectroscopy for numerous trace metals by Mr. George Alexander of the UCLA Department of Nuclear Medicine and Radiation Biology.

Surface slicks and floating particulates

Surface slicks and floating particulates constitute a Bight reservoir that has not received enough research emphasis. Even on a nationwide basis according to the NAS-NAE report "investigations of surface materials of municipal wastewater origin have been extremely scarce . . . Few investigators have attempted to sample and analyze the material actually present on a water surface." SCCWRP added the Southern California region to an existing program funded by the Environmental Protection Agency by collaborating with Dr. R. E. Selleck and Mr. R. Carter of the University of California, Berkeley. Slicks are known to concentrate chemical and biological species at the air-sea interface and may be an important mechanism in the transport of various contaminants to the shoreline or to other areas where they are further concentrated. Preliminary results are available from the first cruise performed on April 7-8, 1971. Sampling was conducted at three locations - directly above the Hyperion five-mile outfall diffuser, two nautical miles down-current from the first station, and in the lee of Santa Catalina Island. Separate surface samples were collected at each station for film materials; floating particulates; and for associated coliform bacteria, neuston, heavy metals, and chlorinated hydrocarbons. The heavy metals and chlorinated hydrocarbons are presently being analyzed. Although derived from a limited number of samples, the findings provide a first quantitative indication of the nature of slicks and floating particulates in the Southern California area. The results suggest that the survival time of coliform bacteria associated with floating particulates may differ substantially from that generally reported for coliforms in sea water.

EFFECTS

Although the chemical inventory discussed above is valuable in determining the geographical areas and organisms on which research emphasis should be placed, the ecological significance of a waste discharge can only be evaluated through biological investigations. SCCWRP presently has four programs underway in this area to serve as a beginning.

External anomalies in fish

SCCWRP has initiated a program specifically addressed to the problem of external anomalies observed in fish collected in Southern California waters. An information search is proceeding to determine the incidence, nature, and possible causes of these anomalies as compared with similar occurrences in Northern California, Oregon, Washington, British Columbia, and Alaska. Emphasis is being placed

on tumors and fin erosion in the dover sole and white croaker. Preliminary results indicate that descriptive terminology is not uniformly employed and numerous contacts with individuals will be necessary to compare the findings. Plans are also being made to coordinate trawling programs and tagging studies involving the sanitation agencies of the region in order to accomplish the following: (a) collect and examine larval and juvenile stages of the fish so that the age and geographical location at which the anomaly first appears may be defined, (b) begin the investigation of the relationships between the presence of an anomaly and all possible causes, including heavy metal or chlorinated hydrocarbon content of the fishes and organic loading of their environment, (c) define local areas where fish pathogens might be transferred to migrating populations, and (d) study the causative factor(s) of fin erosion occurring on fish collected in Southern California waters.

Skeletal deformities and asymmetry in fish

The objective of this study is to develop an accurate and quantitative biological index with which to determine the health of the marine environment. No entirely satisfactory index exists either for the Southern California area or for the coastal zone as a whole. According to the NAS-NAE report (1) "there is a critical need for accurate and simple methods of assessing the general condition of the biota." Skeletal deformities of the sand bass, *Paralabrax nebulifer*, were observed in specimens collected along the Southern California coast. Deformities included asymmetry of ventral fins, gill rakers, opercula, and snout. The SCCWRP-sponsored study will involve the population along the entire Southern California coast and to at least 400 miles below the U.S.-Mexican Border. Preserved specimens are presently being analyzed for age and sex differences, and these parameters will be tested for correlation with deformities. Grunion are also being analyzed for similar deformities.

In addition, the fresh water Japanese rice fish, *Oryzias latipes*, and the fruit fly, *Drosophila melanogaster*, are being raised in the laboratory with exposure to DDT and lead and various other contaminants to investigate the development of asymmetry and in the case of *Drosophila*, the genetic control over asymmetry.

Eutrophication and biostimulation

Another biological index of wastewater effects may be the stimulation of primary productivity, i.e. the photosynthetic formation of carbohydrate by the phytoplankton, in the vicinity of outfall dischargers. In this SCCWRP-sponsored program by Dr. William Thomas, University of California, San Diego, the

following are being investigated: (a) the fraction of nutrients introduced into the Bight as a result of both natural events and human activities; (b) the effects of receiving water samples obtained from sampling stations on laboratory strains of dominant Southern California marine phytoplankters; and (c) the parameters (including sewage) that result in enrichment of natural populations of phytoplankton. Two cruises have been conducted - one on May 4, 1971 and one on June 28-July 2, 1971. Participating in the June-July cruise was Mr. Thor Willsrud of Ventura College. His contribution to the SCCWRP program will provide a basis for the standardization of the fifteen years of data collected by Hyperion personnel on the abundance and variety of plankton in Santa Monica Bay. These data constitute the longest and most continuous record of conditions occurring in the Bay. Bottle and net (similar to the Hyperion net) collections were made at the stations sampled by Dr. Thomas to investigate plankton species diversity and concentration. Net collections when performed alone consistently miss the smaller organisms. As a result of this cruise, it should be possible to correlate data on chlorophyll, productivity, and plankton volume and diversity (from two sampling systems).

Stress and recovery effects of the benthic community

Evaluation of the structure and dynamics of a biological community may lead to another type of environmental health index. According to the NAS-NAE report (1) "The best design would include thorough pre-waste discharge comparison of the disposal site with a similar reference area, and adequate

continuing observation of both after disposal began." Such a project was incorporated into the SCCWRP program to take advantage of the opportunity to study stress and recovery effects around the new and old Orange County outfalls. In cooperation with Dr. E. W. Fager and Mr. Gary Smith, University of California, San Diego, pre- and post-discharge samples have been collected. Although hydrogen sulfide and organic carbon levels have dropped in sediments surrounding the old outfall, no biological effects are yet apparent at either outfall area. The first cruise following the outfall switch in April was on June 21-24, 1971. Future work will include continuing the program of field sampling at both old and new outfall sites and the identification, enumeration, and analysis of invertebrate samples.

SUMMARY

These studies cover only a few of the areas in which much work needs to be done. As an example, a knowledge of the quantity, nature, and origin of constituents entering the coastal waters is vital for control and prediction purposes. However, while pollutant input rates and reservoir concentrations can be measured, the major problem in establishing the ecological significance of treated wastewaters discharged into coastal waters is to establish an index with which to measure their biological impact.

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THE INFLUENCE OF PREVIOUS TREATMENT ON ACCURACY OF MILKFAT ANALYSES DETERMINED ON A MARK III MILKO-TESTER¹

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ABSTRACT

Because of the adaptability of the Milko-Tester to regulatory retesting programs a study was made of the influence of prior treatment on results obtained with a Mark III Milko-Tester. One hundred and two fresh milk samples and 76 composite samples representing 23 different producers were analyzed in duplicate. After initial analysis, samples were cooled and held 5 days before a second analysis. Milkfat was found to average 0.059 and 0.052% lower, respectively, for fresh and composite samples, after storage. The range of differences was, for fresh milk +0.01% to -0.175%, and for preserved samples +0.04% to -0.160%.

Adoption of the Milko-Tester as an official method for milkfat analysis of individual producer's milk supplies, and its adaptability to large scale laboratory testing, brings to question the possibility that the device might be used to retest questionable samples, or be used in a regulatory program as a check-testing method. Research (3, 5, 7, 8) has shown that a number of factors, including free fatty acids, milk homogenized at excessive pressures, sour milk, abnormal milk, and improper mixing cause erratic or imprecise results. Likewise, other investigators (2, 4, 6) have found preservatives to influence Milko-Tester accuracy. Kroger (6) concluded that concentrations of potassium dichromate from 0.08 to 0.80% did not significantly alter milk-fat test up to 3 days' storage at ambient temperature. Decreases of 0.1% and higher occurred on and after the fifth day of storage. He did not retest the same sample, and only one mixed-herd milk supply was analyzed.

There is a trend, now, toward fresh milk testing based upon four samples per month taken on a random stratified basis, one sample from each week of the month. Some states require that samples be cooled after initial testing and then held for a period of days during which the same sample may be picked up and then retested by the regulatory agency. With these concerns in mind, the following work

was done to determine, on a number of mixed-herd milk samples, the influence of prior tempering and testing on Milko-Tester milk fat analyses of both fresh milk and milk preserved with potassium dichromate.

METHODS

During one month's time, farm bulk milk supplies on the regular routes (two every-other-day routes) of a single milk hauler were sampled for this study. Samples were taken in Whirl-Pak bags, held in ice water, and brought to the Dairy Quality Control Institute, Inc. Laboratory for compositing and analysis. A total of 24 producers' supplies were involved.

At the laboratory, samples were tempered for Milko-Tester Mark III analysis in a 100 F tempering bath. When samples reached 100 F, they were mixed by agitating the milk in the bags, a 20-ml sample was taken for compositing, and a portion of the remainder analyzed immediately on a Milko-Tester, Mark III model, Foss Electric Company. The "tested" sample of fresh milk was then cooled in ice water and stored at about 38 F for 5 days before making a second analysis. In all instances, initial testing took place within 24 hr after sample collection. However, of all the samples taken, only four from each producer (one each week for the 4 weeks making up the month) were analyzed on a "fresh" basis.

Composite milk was preserved using 250 mg of potassium dichromate in finished sample volume of 140-160 ml, and analyzed after one 15-day and one 16-day composite period. Composites consisted of milk collected at each pickup interval. After these samples were analyzed initially, they too were cooled in ice water and stored at 38 F for 5 days before retempering for a second analysis. All samples, both fresh and composite, were analyzed in duplicate.

Before the investigation was begun, the Milko-Tester was calibrated and standardized on fresh milk using the Babcock method and the calibration procedure recommended by AOAC (1). It is recognized that the testing of preserved samples requires calibration of the equipment for preserved samples. But this in itself would have no bearing on the absolute differences between initial test results and results obtained on the same sample, be it fresh or preserved composite, after handling and storage, providing no change occurs in calibration.

As an additional check to be certain that samples were properly mixed before testing and compositing, a milk sample from one producer's supply, held and tempered and agitated along with all other samples, was split into three aliquots and each aliquot was tested in duplicate. This pro-

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TABLE 2. RANGE OF DIFFERENCES IN MILKFAT PERCENTAGE AND NUMBER OF SAMPLES IN EACH RANGE LISTING FOR PRIOR TESTED RAW MILK SAMPLES ANALYZED ON A MARK III MILKO-TESTER.

| Range of difference (Percent) | Number of samples | Percent of samples |
|-------------------------------|-------------------|--------------------|
| 0.000 to +0.010 | 6 | 6.7 |
| 0.060 to -0.020 | 12 | 13.5 |
| -0.021 to -0.040 | 10 | 11.2 |
| -0.041 to -0.060 | 22 | 24.7 |
| -0.061 to -0.080 | 18 | 20.2 |
| -0.081 to -0.100 | 9 | 10.1 |
| -0.101 to -0.120 | 6 | 6.7 |
| -0.121 to -0.140 | 3 | 3.4 |
| -0.141 to -0.160 | 2 | 2.3 |
| -0.161 to -0.180 | 1 | 1.2 |

cedure was followed on every other day of collection. The range of difference between these check samples varied from no difference (on one sample) to as high as 0.065% (also on one sample only) and averaged, over the month, 0.0069%. This indicates control in sampling and testing well within accepted limits for official analyses of milk for producer payment.

RESULTS AND DISCUSSION

In Table 1 may be seen the differences by sample and by producer for four raw milk samples analyzed once, then cooled and held 5 days before retempering and retesting. Of a total of 89 samples of milk, only four showed higher analytical values upon re-

testing, and then to only a very slight degree (0.005% to 0.010%). Two samples showed no difference in milk fat values, and 83, the remainder, showed a negative relationship. Overall, the range of difference varied from 0.01% to -0.175% and averaged -0.059%. Also, it should be noted that differences within an individual milk supply were not very consistent. From week to week, samples were found to vary considerably in the apparent effect of prior treatment on test results. While these values are averages of duplicates, and single test values might be expected to differ more widely, in no instance did duplicate tests vary by more than 0.04%. Thus, the averaged values represent closely the widest fluctuations that might be expected considering even single test observations.

These data are segregated incrementally into various range categories in Table 2. The majority of samples, 62 of the 89, varied upon retesting between 0.0% and -0.080%. Negative values predominate and several fall outside the 0.1% range.

As shown in Table 3, much the same trend holds for composited, preserved samples of milk as for fresh milk. Once again, prior tempering and storage resulted in generally lower test values. Some samples varied positively and to a slightly greater magnitude (0.020% to 0.060%) then was observed on fresh samples. Still, negative differences predominated and

TABLE 1. INFLUENCE OF PRIOR TESTING ON MILKFAT ANALYSES OF RAW MILK ANALYZED ON A MARK III MILKO-TESTER

| Producer number | Average ¹ milkfat percentages | | | | | | | | | | | | Avg. Diff. | |
|--------------------------|--|--------------------|--------|----------|-------|--------|----------|-------|--------|----------|-------|--------|------------|--------|
| | Sample 1 | | | Sample 2 | | | Sample 3 | | | Sample 4 | | | | |
| | Initial ² | 5-day ² | Diff. | Initial | 5-Day | Diff. | Initial | 5-Day | Diff. | Initial | 5-Day | Diff. | | |
| 1 | 3.420 | 3.365 | -0.055 | 3.475 | 3.390 | -0.085 | Sample | Not | Taken | Sample | Not | Taken | -0.070 | |
| 2 | 3.550 | 3.495 | -0.055 | 3.495 | 3.430 | -0.065 | 3.610 | 3.520 | -0.090 | 3.575 | 3.515 | -0.060 | -0.060 | |
| 3 | 3.815 | 3.765 | -0.110 | 3.655 | 3.620 | -0.035 | 3.690 | 3.625 | -0.065 | 3.730 | 3.715 | -0.015 | -0.056 | |
| 4 | 3.760 | 3.710 | -0.050 | 3.645 | 3.540 | -0.145 | 3.695 | 3.595 | -0.100 | Sample | Not | Taken | -0.083 | |
| 5 | 5.005 | 4.935 | -0.070 | 4.760 | 4.585 | -0.175 | 4.945 | 4.865 | -0.080 | 4.725 | 4.620 | -0.105 | -0.107 | |
| 6 | 3.350 | 3.350 | 0.000 | 3.475 | 3.430 | -0.045 | 3.370 | 3.355 | -0.015 | 3.475 | 3.485 | +0.010 | -0.017 | |
| 7 | 3.465 | 3.400 | -0.065 | 3.350 | 3.290 | -0.060 | 3.690 | 3.630 | -0.060 | 3.505 | 3.455 | -0.050 | -0.058 | |
| 8 | 3.800 | 3.735 | -0.065 | 3.865 | 3.755 | -0.110 | 3.765 | 3.700 | -0.065 | 3.925 | 3.875 | -0.050 | -0.072 | |
| 9 | 3.100 | 3.090 | -0.010 | 3.280 | 3.205 | -0.075 | 3.240 | 3.205 | -0.035 | 3.280 | 3.265 | -0.015 | -0.033 | |
| 10 | 3.730 | 3.680 | -0.050 | 3.895 | 3.770 | -0.125 | 3.850 | 3.775 | -0.075 | 3.775 | 3.740 | -0.035 | -0.071 | |
| 11 | 3.395 | 3.355 | -0.040 | 3.370 | 3.260 | -0.110 | 3.445 | 3.395 | -0.045 | 3.465 | 3.425 | -0.040 | -0.058 | |
| 12 | 3.915 | 3.815 | -0.100 | 3.905 | 3.800 | -0.105 | 3.720 | 3.645 | -0.075 | 3.640 | 3.593 | -0.047 | -0.081 | |
| 13 | 3.735 | 3.635 | -0.100 | 3.785 | 3.735 | -0.055 | 3.700 | 3.640 | -0.140 | 3.710 | 3.615 | -0.095 | -0.097 | |
| 14 | 3.990 | 3.930 | -0.070 | 3.950 | 3.930 | -0.020 | 4.600 | 3.920 | -0.080 | 4.050 | 3.940 | -0.110 | -0.070 | |
| 15 | 3.665 | 3.580 | -0.085 | 3.555 | 3.565 | +0.010 | 3.625 | 3.580 | -0.045 | 3.600 | 3.520 | -0.080 | -0.055 | |
| 16 | 3.505 | 3.425 | -0.080 | 3.455 | 3.455 | 0.000 | 3.420 | 3.375 | -0.045 | 3.530 | 3.435 | -0.045 | -0.042 | |
| 17 | 3.685 | 3.670 | -0.015 | 3.740 | 3.735 | -0.005 | 3.745 | 3.695 | -0.050 | 3.715 | 3.625 | -0.090 | -0.040 | |
| 18 | 3.875 | 3.830 | -0.045 | 3.835 | 3.815 | -0.020 | 3.805 | 3.780 | -0.025 | 3.775 | 3.630 | -0.145 | -0.058 | |
| 19 | 4.130 | 4.060 | -0.070 | 4.255 | 4.220 | -0.035 | 4.225 | 4.180 | -0.045 | 4.135 | 4.055 | -0.080 | -0.057 | |
| 20 | 3.715 | 3.700 | -0.015 | 3.750 | 3.760 | +0.010 | 3.805 | 3.790 | -0.015 | 3.805 | 3.705 | -0.100 | -0.035 | |
| 21 | 3.820 | 3.760 | -0.060 | 3.775 | 3.735 | -0.040 | 3.770 | 3.750 | -0.020 | 3.780 | 3.655 | -0.125 | -0.061 | |
| 22 | 3.205 | 3.175 | -0.030 | 3.425 | 3.430 | +0.005 | 3.450 | 3.430 | -0.020 | 3.390 | 3.315 | -0.075 | -0.032 | |
| 23 | 3.585 | 3.535 | -0.050 | 3.575 | 3.515 | -0.060 | 3.670 | 3.640 | -0.030 | 3.655 | 3.580 | -0.075 | -0.053 | |
| Range = +0.010 to -0.175 | | | | | | | | | | | | | Grand Avg. | -0.059 |

¹Values are averages of duplicate analyses made on raw milk samples collected once each week on four consecutive weeks.
²Initial testing was done within 24 hours after samples were picked up by the milk hauler. Samples were then cooled in ice water and stored five days prior to re-testing.

TABLE 3. INFLUENCE OF PRIOR TESTING ON MILKFAT PERCENTAGES¹ OF 15- AND 16-DAY COMPOSITED RAW MILK SAMPLES PRESERVED WITH POTASSIUM DICHROMATE AND ANALYZED ON A MARK III MILKO-TESTER.

| Producer number | Milkfat percentages | | | | | | | Avg. Diff. | | |
|-----------------------|---------------------|---------|--------|------------------|---------|--------|-------|------------------|--------|--------|
| | 16-Day composite | | | 15-Day composite | | | | | | |
| | Initial Test | Re-Test | Diff. | Initial Test | Re-Test | Diff. | | | | |
| 1 | 3.430 | 3.455 | +0.025 | 3.250 | 3.285 | +0.035 | | +0.030 | | |
| 2 | 3.540 | 3.475 | -0.065 | 3.465 | 3.440 | -0.025 | | -0.045 | | |
| 3 | 3.670 | 3.605 | -0.065 | 3.655 | 3.610 | -0.045 | | -0.055 | | |
| 4 | 3.670 | 3.595 | -0.075 | 3.675 | 3.595 | -0.080 | | -0.077 | | |
| 5 | 4.765 | 4.640 | -0.125 | 4.645 | 4.475 | -0.170 | | -0.147 | | |
| 6 | 3.420 | 3.440 | +0.020 | 3.345 | 3.390 | +0.060 | | +0.040 | | |
| 7 | 3.480 | 3.420 | -0.060 | 3.500 | 3.400 | -0.100 | | -0.035 | | |
| 8 | 3.750 | 3.675 | -0.075 | 3.755 | 3.625 | -0.130 | | -0.102 | | |
| 9 | 3.220 | 3.185 | -0.035 | 3.260 | 3.240 | -0.020 | | -0.027 | | |
| 10 | 3.655 | 3.530 | -0.125 | 3.695 | 3.570 | -0.125 | | -0.125 | | |
| 11 | 3.355 | 3.325 | -0.030 | 3.350 | 3.275 | -0.075 | | -0.052 | | |
| 12 | 3.885 | 3.810 | -0.075 | 3.685 | 3.595 | -0.090 | | -0.082 | | |
| 13 | 3.810 | 3.650 | -0.160 | 3.780 | 3.640 | -0.140 | 3.710 | 3.615 | -0.092 | -0.097 |
| 14 | 3.950 | 3.905 | -0.045 | 3.970 | 3.845 | -0.125 | | | -0.085 | |
| 15 | 3.650 | 3.615 | -0.035 | 3.560 | 3.525 | -0.035 | | | -0.035 | |
| 16 | 3.435 | 3.415 | -0.020 | 3.445 | 3.425 | -0.020 | | | -0.020 | |
| 17 | 3.610 | 3.605 | -0.005 | 3.600 | 3.580 | -0.020 | | | -0.012 | |
| 18 | 3.785 | 3.785 | 0.000 | 3.740 | 3.675 | -0.065 | | | -0.032 | |
| 19 | 4.105 | 4.020 | -0.085 | 4.140 | 3.995 | -0.145 | | | -0.115 | |
| 20 | 3.695 | 3.720 | +0.025 | 3.735 | 3.705 | -0.030 | | | -0.003 | |
| 21 | 3.685 | 3.610 | -0.075 | 3.650 | 3.555 | -0.095 | | | -0.085 | |
| 22 | 3.200 | 3.220 | +0.020 | 3.350 | 3.395 | +0.045 | | | +0.032 | |
| 23 | 3.555 | 3.535 | -0.020 | 3.600 | 3.575 | -0.025 | | | -0.022 | |
| Grand Avg. Difference | | | | | | | | -0.052 | | |
| Range = | | | | | | | | +0.060 to -0.160 | | |

¹Samples were analyzed on a Mark III Milko-Tester at the end of the composite period. They were then cooled in ice water, and re-tested after five additional days of refrigerated storage. All samples were analyzed in duplicate.

ranged as low as -0.170%. The grand average difference was -0.052%, not greatly different than the -0.059% noted on fresh samples. However, between samples from the same producers, there does appear to be a slightly better consistency. Table 4 shows the breakdown by various ranges of difference, and again the majority (27 of 46) fall into the range between 0.0 and -0.080%. These results agree closely with the findings of Kroger (6).

Taken as a whole, the data indicate the potential

TABLE 4. RANGE OF DIFFERENCES IN MILKFAT PERCENTAGE AND NUMBER OF SAMPLES IN EACH RANGE LISTING FOR PRIOR TESTED COMPOSITE MILK SAMPLES ANALYZED ON A MARK III MILKO-TESTER.

| Range of difference (Percent) | Number of samples | Percent of samples |
|-------------------------------|-------------------|--------------------|
| +0.041 to +0.060 | 2 | 4.4 |
| +0.021 to +0.040 | 3 | 6.5 |
| 0.000 to +0.020 | 2 | 4.4 |
| 0.000 to -0.020 | 8 | 17.4 |
| -0.021 to -0.040 | 5 | 15.2 |
| -0.041 to -0.060 | 3 | 6.5 |
| -0.061 to -0.080 | 9 | 19.6 |
| -0.081 to -0.100 | 3 | 6.5 |
| -0.101 to -0.120 | 0 | 0.0 |
| -0.121 to -0.140 | 5 | 10.9 |
| -0.141 to -0.160 | 3 | 6.5 |
| -0.161 to -0.180 | 1 | 2.2 |

for test variations when fresh or preserved samples are reheated and retested on a Mark III Milko-Tester. While other test methods may show equal or greater variability under similar test conditions, it seems important, particularly where legal questions may arise, to know the limits of automated methods which lend themselves so well to mass testing.

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LACTOSE HYDROLYSIS IN MILK AND MILK PRODUCTS BY BOUND FUNGAL BETA-GALACTOSIDASE

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ABSTRACT

The β -galactosidase of *Aspergillus niger* was immobilized by glutaraldehyde coupling to porous glass beads and the bound enzyme evaluated for its applicability to hydrolysis of lactose in milk and milk products. Lactose in sweet whey and skim milk was hydrolyzed at approximately one-third the rate in acid whey. Non-lactose solids inhibited β -galactosidase activity. Greater efficiency of lactose hydrolysis was obtained with the bound enzyme in column operations than in stirred batch reactors.

Use of β -galactosidases to hydrolyse lactose in milk and milk products is a subject of renewed interest (1, 7, 8). Applications are readily apparent not only for the food industry, but also to prepare low-lactose dairy products intended for use by lactose intolerant individuals. Nonfat dry milk and whey powder products with portions of their lactose hydrolyzed have been prepared with good flavor, appearance, and stability (7).

The purity, availability, and cost of β -galactosidases become important economic considerations in any large scale lactose-hydrolysis process. Although satisfactory hydrolysis is obtainable through addition of free enzymes, their one-time use appears uneconomical. Recent developments in enzyme immobilization (3) permit continuous extended use of the bound enzymes and can reduce costs significantly.

A comparative evaluation of microbial β -galactosidases indicates that the fungal enzyme appears more suited for use in immobilized systems than the yeast or bacterial enzymes. The *Aspergillus niger* β -galactosidase (β -D-galactoside galactohydrolase EC 3.2.1.23) offers several distinct advantages, namely, an operational half-life of perhaps several months, high heat stability, and freedom from metal requirements. Admittedly, use of the enzyme (pH optimum 4.0) appears to be limited primarily to acid whey products since skim milk (pH 6.8) and rennet wheys (pH 6.3) fall on the low end of its effective pH range. Further, the competitive inhibition exerted by the liberated galactose (9) markedly diminishes the efficiency of the enzyme during the continuous hydrolysis of lactose. Nevertheless, the applicability of immobilized *A. niger* β -galactosidase for the hydro-

lysis of lactose in several milk products will be evident from data in this report.

MATERIALS AND METHODS

β -Galactosidase

A partially purified preparation of the β -galactosidase of *A. niger* (Lactase LP) was a gift of Wallerstein Laboratories and was used without further purification.

Preparation of bound enzymes

The enzyme preparations were attached to Corning's Controlled Pore² glass beads (pore size $700 \pm 70A$, 120-200 mesh) by glutaraldehyde crosslinking according to the procedure reported by Robinson et al. (5). The glass beads were immersed in a 2% solution of 3-aminopropyltriethoxysilane in acetone for 24 hr at 45 C. Aminoalkylsilane glass was stirred in a cold 1% aqueous solution of glutaraldehyde for 30 min, rinsed with water, and then suspended in cold phosphate buffer (pH 7.5) containing the β -galactosidase. After 2 hr the glass was rinsed with several volumes of H₂O, 0.5 M NaCl, and finally with 0.1 M acetate buffer, pH 4. No soluble β -galactosidase activity could be eluted from the glass following the washing procedures. Protein analysis of the supernatant and rinses by the Lowry procedure (4) indicated the coupling of 7 mg protein/g of glass. This preparation was used for the experiments reported below.

Enzyme activity and lactose hydrolysis

Enzymatic activity was determined by the amount of glucose released following incubation of either the free or bound β -galactosidases with substrate. Aliquots of digests containing the free enzyme were placed in a boiling water bath for 3 min to stop the activity before assay for glucose content. Activities of the immobilized enzymes were determined by pumping substrate through columns of bound enzyme or by incubation with a suspension maintained by overhead stirring. Appropriate aliquots were analyzed directly for glucose content by a glucose oxidase procedure (2) calibrated with standard glucose concentrations.

Skimmilk and whey samples

Raw skimmilk was obtained from a commercial dairy; the rennet and acid wheys were prepared by laboratory procedures. Products were pasteurized by holding at 65 C for 30 min.

RESULTS AND DISCUSSION

Although use of β -galactosidase preparations obtained by affinity chromatography (6, 9) would per-

¹Agricultural Research Service, U. S. Department of Agriculture.

²Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. THE EFFECT OF FLOW-RATE ON THE HYDROLYSIS OF LACTOSE IN VARIOUS LACTOSE-CONTAINING SUBSTRATES¹

| Substrate ² | Lactose hydrolyzed (%) | |
|------------------------|------------------------|----------|
| | 70 ml/hr | 35 ml/hr |
| 5% Lactose, pH 4.7 | 49 | 65 |
| Acid whey, pH 4.7 | 43 | 61 |
| Rennet whey, pH 6.6 | 15 | 24 |
| Skim milk, pH 6.6 | 12 | 23 |

¹A 4-cc column of bound enzyme operating at 37 C.

²Lactose content adjusted to 5% in all samples.

TABLE 2. INFLUENCE OF NON-LACTOSE ACID WHEY SOLIDS ON THE RATE OF LACTOSE HYDROLYSIS¹

| Non-lactose solids (%) | Lactose hydrolyzed ² (%) |
|------------------------|-------------------------------------|
| 3.8 | 24.1 |
| 2.5 | 24.9 |
| 1.5 | 27.2 |
| 1.0 | 29.7 |

¹A 4-cc column of bound enzyme operating at 100 ml/hr at 37 C.

²Lactose content adjusted to 10% in all samples.

TABLE 3. INFLUENCE OF SOLIDS CONTENT ON LACTOSE HYDROLYSIS IN ACID WHEY¹

| Total solids (%) | Lactose hydrolyzed (%) |
|------------------|------------------------|
| 5 | 22.2 |
| 10 | 17.8 |
| 20 | 12.6 |
| 30 | 9.1 |

¹A 4-cc column of bound enzyme operating at 100 ml/hr at 37 C.

mit the binding of more enzymatic activity to the glass, it was believed important to evaluate an enzyme preparation as commercially available. The specific activity of the partially purified preparation was half that of the affinity purified *A. niger* β -galactosidase. The β -galactosidase was bound to the alkylamine glass using the glutaraldehyde procedure which is substantially simpler and more rapid than diazo coupling (9).

The bound enzyme had the same functional and stability properties as the free enzyme and retained approximately 75% of its original activity. The pH activity curve in Fig. 1a shows that the enzyme at pH 4.5 possesses 80% of its optimum activity, but only 10% at pH 6.5. Thus the relative efficiency of the enzyme for hydrolysis of lactose in milk products decreases significantly above pH 5.0. The effect of temperature on enzymatic activity is shown in Fig. 1b. The fact that maximum activity occurs at 55 C offers an advantage in that operations at that temperature would be essentially free of most microbial growth.

As was shown in another publication (9), galactose is a competitive inhibitor of the *A. niger* β -galactosidase. This inhibition increased during the course of lactose hydrolysis and substantially reduced the enzyme efficiency. For example, 0.01 M galactose causes a 60% inhibition in the hydrolysis of 0.1 M lactose. Thus, the hydrolysis rate is not proportional to the amount of enzyme added or to the time of exposure. This is readily apparent from data in Table 1 which show the effect of flow rate on hydrolysis

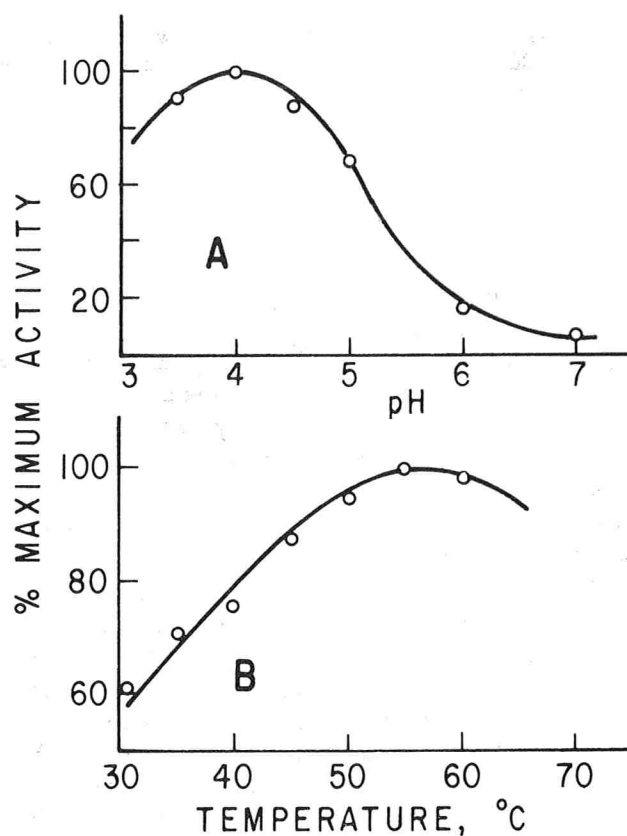


Figure 1. Influence of pH (A) and temperature (B) on the activity of *A. niger* β -galactosidase.

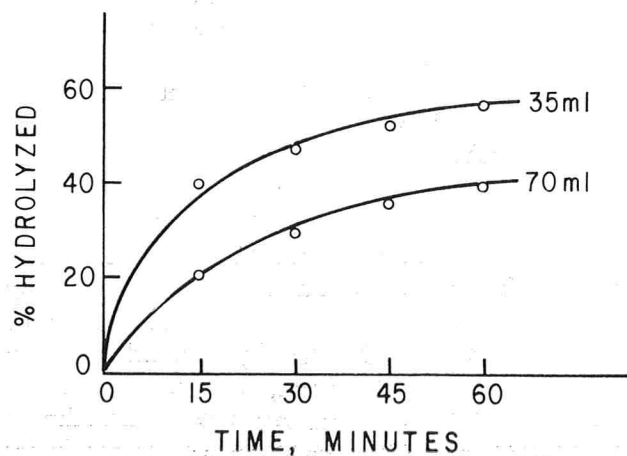


Figure 2. Influence of substrate volumes on the hydrolysis rates of 5% lactose (pH 4, 37°C) by stirred enzyme suspensions.

of various lactose containing substrates by a 4-cc column of immobilized enzyme. Doubling the enzyme exposure time of skim milk and sweet whey increased the hydrolysis rate 60-90%. However, because of the increased amounts of lactose hydrolyzed, and consequently the greater galactose inhibition, the increase did not exceed 42% in the acid products.

The extent of lactose hydrolysis in the substrates shown in Table 1 was dependent largely on their pH, with the neutral substrates being hydrolyzed at approximately one-third the rate of the acid substrates. It appeared that the non-lactose solids had an effect on the hydrolysis rates. This is most evident in the comparison between the values obtained for 5% lactose and acid whey. This effect was investigated further by determining lactose hydrolysis rates at various concentrations of non-lactose solids. Lyophilized acid whey was reconstituted to various concentrations and the lactose content of all samples adjusted to 10%. The effects on the hydrolysis rates were determined with a 4-cc column of immobilized enzyme. Data presented in Table 2 show that the hydrolysis rates increased as the solids content decreased. These results are similar to data obtained by Wendorf et al. (7) using solutions of *Saccharomyces fragilis* β -galactosidase. Data in Table 3 show that as the total solids increase in whey concentrates, increased amounts of lactose are hydrolyzed, although the percent of total lactose hydrolyzed decreases.

Immobilized enzymes can be used either batchwise or in column operations. However, with β -galactosidase, more efficient lactose hydrolysis was obtained by continuous-flow column operation compared to batch treatments with stirred suspensions of immobilized enzyme. The influence of substrate volumes on the hydrolysis rates of 5% lactose (pH 4, 37 C) by stirred enzyme suspensions is shown in Fig. 2. At the end of 1 hr, 56% lactose hydrolysis was obtained with 35 ml of substrate and 39% with 70 ml. In a column operation, the same 4-cc of bound en-

zyme hydrolyzed 73% of the lactose at 35 ml/hr and 53% at 70 ml/hr. These differences in amounts of lactose hydrolyzed can be attributed to the fact, that in a batch operation, all of the enzyme is exposed to increasing levels of galactose inhibition whereas, in a column operation, part of the enzyme is operating at higher substrate concentrations and lower levels of galactose inhibition.

A comparison of the lactose hydrolysis rates in raw and pasteurized acid wheys, skimmilks, and whole milks indicated that no inhibition of enzyme activity was operative in the raw samples compared with those pasteurized at 65 C for 30 min. This is in contrast to the reported inhibition of the β -galactosidase of *S. fragilis* by raw milk products. (7).

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ISOLATION OF SALMONELLA FROM CARCASSES OF STEAM- AND WATER-SCALDED POULTRY

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ABSTRACT

This study was made to compare the effectiveness of two methods of scalding in reducing *Salmonella* surface contamination on carcasses of broilers processed under commercial conditions. The number of chickens contaminated with *Salmonella* approximately doubled between scalding and defeathering and between defeathering and chilling. More than two times as many positive samples were found on water-scalded birds as on steam-scalded birds. Of 348 carcasses sampled from each method of scalding, 27 water-scalded carcasses and 12 steam-scalded carcasses were contaminated with *Salmonella*. Seven serotypes were found on water-scalded poultry, only three were found on steam-scalded poultry. In this study, steam scalding was more effective (although not statistically significant) than conventional hot water scalding in reducing the number of chicken carcasses contaminated with *Salmonella*. *Salmonella* surface contamination present after scalding may be a source of subsequent contamination of other carcasses during processing operations through chilling.

Salmonellosis is primarily an animal disease but human illness results from ingesting the contaminated animal product. Since poultry is a major food source, *Salmonella* contamination of poultry is of great concern to the industry as both an economic and a public health problem. Market poultry has been found to be contaminated with *Salmonella* (1, 3, 4, 5). An effective method of processing that would reduce the presence of *Salmonella* on carcasses of contaminated poultry would be of value to the poultry industry. Steam scalding has been reported to be more effective than hot water scalding in reducing total bacteria and coliform counts on carcasses of commercially processed broilers (6, 7). The objective of this study was to compare the effectiveness of a prototype steam scalding and a conventional hot water scalding in reducing *Salmonella* surface contamination on carcasses of broilers processed under commercial conditions.

EXPERIMENTAL PROCEDURE

Broilers used in this study were taken from the processing line at a commercial broiler plant. One-half of the birds were scalded at 55 C for 2 min using a conventional hot water scalding, while the other half were scalded at 53-55 C for 2.25 min with a steam-hot water spray using a prototype of a steam scalding. Each scalding unit was operated in parallel but on a separate defeathering and eviscerating line. Chickens from the same flock entered the two scalding lines simultaneously. Samples were taken for 5 days during a two-week period.

Swab samples, using standard techniques, were taken from

the adjacent areas of the right thigh at three different positions along the line for each type of scalding; namely immediately after scalding, Position 1; after defeathering, Position 2; and upon exiting from the continuous chiller, Position 3. Samples obtained at Position 1 required hand removal of the feathers so the skin was exposed for swabbing. An area 1.88 cm² was swabbed using a glass template to give a clearly defined area. Two swab samples were taken from each bird with one swab sample being placed in a screw cap tube containing 10 ml of freshly prepared tetrathionate broth (Difco) and the other swab in 10 ml of selenite cystine broth (Difco). Five birds from each scalding line were sampled at 7:00, 9:00 and 11:00 a.m. and at 2:00 and 4:00 p.m. to test for possible build-up of *Salmonella* in the plant. Collected samples were transported to the laboratory (within 5 hr of sampling), incubated 48 hr at 37 C and streaked on salmonella-shigella (SS) agar (Difco) and bismuth sulfite (BS) agar (BBL). SS agar plates showing no growth or all lactose fermenting colonies after 24 hr of incubation at 37 C were discarded. BS agar plates were examined for growth after 24 and 48 hr of incubation. Plates showing no growth after 48 hr were considered negative and were discarded. At least two colonies were picked into brain heart infusion (BHI) broth from remaining plates, and incubated at 37 C for 24 hr. Cultures were then purified by streaking onto MacConkey agar (Difco) and incubated at 37 C for 24 hr. Plates showing only lactose fermenting colonies on MacConkey agar were discarded.

After purification, all isolates were inoculated into Triple-Sugar-Iron (TSI) agar (Difco) and screened biochemically according to methods of Galton et al. (2). All typical *Salmonella* cultures were tested serologically using Bacto-Salmonella H antiserum poly A-Z, and then were submitted to the Center for Disease Control in Atlanta for serotyping.

RESULTS AND DISCUSSION

Samples were taken periodically throughout each of 5 days to check for *Salmonella* build-up on equipment. The smallest number of *Salmonella*-contaminated carcasses obtained from water-scalded poultry was obtained at the 7:00 a.m. and 2:00 p.m. sampling times. These times were shortly after the plant began operation in the morning and after clean-up during the lunch period. The greatest concentration of isolates were found at 9:00 a.m. Fewer steam-scalded chickens were contaminated with *Salmonella* than were water-scalded chickens, but some build-up in environmental contamination did occur on the steam line in the afternoon.

Data in Table 1 give the number of *Salmonella* isolates from each sampling station for the two scald-

TABLE 1. SALMONELLA ISOLATED FROM STEAM AND WATER SCALDED BROILERS DURING FIVE DAYS OF SAMPLING

| Day | Station | Water scald | | | Serotype | Steam scald | | | |
|--------|---------|-------------------|----------------------------|---------------------------|--|-------------------|----------------------------|---------------------------|---|
| | | No. birds sampled | No. with <i>Salmonella</i> | Percent <i>Salmonella</i> | | No. birds sampled | No. with <i>Salmonella</i> | Percent <i>Salmonella</i> | Serotype |
| 1 | 1 | 16 | 0 | 0 | | 16 | 1 | 6.3 | <i>infantis</i> |
| | 2 | 16 | 2 | 12.5 | <i>typhimurium</i> <i>var. copenhagen</i> (2) | 16 | 1 | 6.3 | <i>infantis</i> |
| | 3 | 16 | 3 | 18.8 | <i>typhimurium</i> <i>var. copenhagen</i> (3) | 16 | 0 | 0 | |
| Totals | | 48 | 5 | 10.4 | | 48 | 2 | 4.2 | |
| 2 | 1 | 25 | 0 | 0 | | 25 | 0 | 0 | |
| | 2 | 25 | 0 | 0 | | 25 | 0 | 0 | |
| | 3 | 25 | 1 | 4 | <i>infantis</i> | 25 | 0 | 0 | |
| Totals | | 75 | 1 | 1.3 | | 75 | | | |
| 3 | 1 | 25 | 0 | 0 | | 25 | 0 | 0 | |
| | 2 | 25 | 0 | 0 | | 25 | 1 | 4 | <i>infantis</i> |
| | 3 | 25 | 2 | 8 | <i>blegdam</i> (1) <i>enteriditis</i> (1) | 25 | 0 | 0 | |
| Totals | | 75 | 2 | 2.7 | | 75 | 1 | 1.3 | |
| 4 | 1 | 25 | 1 | 4 | <i>enteriditis</i> | 25 | 0 | 0 | |
| | 2 | 25 | 1 | 4 | <i>infantis</i> | 25 | 0 | 0 | |
| | 3 | 25 | 1 | 4 | <i>infantis</i> | 25 | 0 | 0 | |
| Totals | | 75 | 3 | 4 | | 75 | | | |
| 5 | 1 | 25 | 4 | 16 | <i>newport</i> (1) <i>infantis</i> (3) | 25 | 0 | 0 | |
| | 2 | 25 | 4 | 16 | <i>infantis</i> (2) <i>anatum</i> (1) <i>typhimurium</i> <i>var. copenhagen</i> (1) | 25 | 2 | 8 | <i>infantis</i> (2) |
| | 3 | 25 | 8 | 32 | <i>infantis</i> (1) <i>newport</i> (5) <i>thompson</i> (2) | 25 | 7 | 28 | <i>newport</i> (1) <i>thompson</i> (6) |
| Totals | | 75 | 16 | 21.3 | | 75 | 9 | 12 | |

() = number of isolates

ing methods. The number of *Salmonella* isolates found varied greatly between days on which samples were taken, ranging from one isolate on day two to 25 on day five. This variation would be expected because of differences in levels of *Salmonella* in different flocks.

The number of *Salmonella*-contaminated carcasses obtained from each station over a 5-day sampling period is given in Table 2 along with the serotype found. At station one, 5 of 116 (4.3%) water-scalded birds had *Salmonella* compared to only 1 of 116 (0.9%) for the steam-scalded birds. At station two, 7 of 116 (6%) of the water-scalded birds were found contaminated compared to 4 of 116 (3.4%) steam-scalded birds. After chilling at station three, the number of water-scalded birds having *Salmonella* increased to 15 of 116 (12.9%) birds, and the steam-scalded birds rose to 6 of 116 (5.2%). The number of positive carcasses increased at each sampling station as carcasses moved through the process lines of each scald.

The increase in numbers of *Salmonella* isolates found at station two agrees with the results of Galton

and co-workers (1) who observed that 16 of 20 samples taken from defeathering machines in a duck processing plant yielded *Salmonella*. The large number of *Salmonella* found at station three is in agreement with the finding of Morris and Wells (5) who reported that contamination of chicken carcasses occurred during evisceration and chilling, areas of extensive handling, and extensive contact among birds.

In this study more water-scalded chickens were found to be contaminated with *Salmonella* than were steam-scalded chickens. A total of 27 of 348 (7.8%) water-scalded birds were contaminated with *Salmonella* compared to 12 of 348 (3.4%) of the steam-scalded birds.

A randomized block chi-square test showed no statistically significant difference between the two scald methods at either of the three stations. Lack of statistical significance resulted from an insufficient number of samples.

A total of seven *Salmonella* serotypes were found in this study (Table 2). Of this total, three serotypes were obtained from birds at Station 1, two

TABLE 2. SALMONELLA ISOLATES FROM STEAM AND WATER SCALDED BROILERS AT DIFFERENT STAGES OF PROCESSING

| Station | Water scald | | | Serotypes | Steam scald | | | Serotypes |
|---------|-------------|----------------------------|---------------------------|--|-------------|----------------------------|---------------------------|---|
| | No. birds | No. with <i>Salmonella</i> | Percent <i>Salmonella</i> | | No. birds | No. with <i>Salmonella</i> | Percent <i>Salmonella</i> | |
| 1 | 116 | 5 | 4.3 | <i>enteritidis</i> (1) <i>infantis</i> (3) <i>newport</i> (1) | 116 | 1 | 0.86 | <i>infantis</i> (1) |
| 2 | 116 | 7 | 6.0 | <i>typhimurium</i> var. <i>copenhagen</i> (3) <i>infantis</i> (3) <i>anatum</i> (1) | 116 | 4 | 3.4 | <i>infantis</i> (4) |
| 3 | 116 | 15 | 12.9 | <i>typhimurium</i> var. <i>copenhagen</i> (3) <i>infantis</i> (3) <i>blegdam</i> (1) <i>enteritidis</i> (1) <i>newport</i> (5) <i>thompson</i> (2) | 116 | 7 | 6 | <i>newport</i> (1) <i>thompson</i> (6) |

() = number of isolates

TABLE 3. SALMONELLA SEROTYPES ISOLATED DURING THIS STUDY

| Order of frequency | <i>Salmonella</i> serotype | No. isolated |
|--------------------|---|--------------|
| 1 | <i>S. infantis</i> ^a | 14 |
| 2 | <i>S. thompson</i> ^a | 8 |
| 3 | <i>S. newport</i> ^a | 7 |
| 4 | <i>S. typhimurium</i> var. <i>copenhagen</i> ^a | 6 |
| 5 | <i>S. enteritidis</i> ^a | 2 |
| 6 | <i>S. anatum</i> ^a | 1 |
| 7 | <i>S. blegdam</i> | 1 |

^aThese six serotypes were listed among the 14 most common serotypes from chicken origin reported in 1970. These were reported to the Center for Disease Control in Atlanta, Georgia.

serotypes were isolated at Station 2, while six serotypes were found at Station 3.

Data in Table 3 lists the *Salmonella* serotypes found in this study and their rank in frequency of occurrence in chickens. All isolates were among the 14 most common serotypes found in 1970 with the exception of *Salmonella blegdam* (8). Four of the serotypes reported in this study, *Salmonella typhimurium* var. *copenhagen*, *Salmonella enteritidis*, *Salmonella anatum*, and *S. blegdam* were not isolated from steam-scalded birds. These four serotypes represent 25% of the contaminated carcasses.

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LAGOONS: A TREATMENT FOR MILKING CENTER WASTE¹

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ABSTRACT

Operational data from six lagoons treating milking center (milkhouse and milking parlor) waste were collected in New York State during 1971-72. Few advantages occur from using settling basins or expensive out fall structures. Design criteria fail to recognize that individual site geography can appreciably vary a pond's winter snow load from those projected from regional averages. Individual farm management practices make the difference between success or failure of a milk waste system. Dairymen can capture the resources currently being misplaced from milking center effluents by seasonally applying them to crops and land.

This study was made to determine how naturally aerated lagoons in New York State perform with milking center wastes. Lagoons have been used in the dairy industry to treat high strength wastes such as cheese whey or effluents from butter-powder processing plants and others. The literature is extensive with documents describing lagoon experiences with industrial and domestic wastes. Harper (2) in 1971 summarized information on the dairy industry's waste practices in his monumental state of the art report.

Zall (3) elucidated some characteristics of milking center waste in his 1971 study of 24 New York State dairy farms. One type of waste treatment method has been examined in this investigation. In addition, this study corroborates the validity of New York's lagoon design criteria as initially established by regulatory and university personnel.

Currently, basic specifications for building lagoons for milking center wastes are: 125 ft² of surface area/cow calculated at a 2-ft. level; the system must contain an in-line settling basin which is sized at 18 gal per cow; the pond's volume will hold 400 days' waste calculated at 6 gal/cow/day. Liquid level is to be maintained between 2-5 ft; an outlet structure shall be similar to damming devices or appropriate valves. Crowe (1) reported the rationale for these guidelines in 1972.

MATERIALS AND METHODS

From September, 1971 through August, 1972 researchers

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monitored six treatment lagoons monthly with on-site visits. Duplicate samples were collected at pond centers and at different levels. One sample set was field tested for dissolved oxygen while the second set was transported in styrofoam picnic baskets to the Cornell campus laboratories where testing was done. Biological and chemical analysis followed the procedures outlined in the 1971 Environmental Protection Agency Water Quality office, Methods for Chemical Analysis of Water and Wastes.

During the winter, pond sampling was made easy by ice formation. Extension specialists chopped holes at appropriate locations and used a multi-level sampling staff with fixed sampling bottle locations to gather lagoon fluids. As weather warmed and ice melted, a two-man rubber boat was used as a floating platform to obtain samples. Several areas in the ponds were tested to determine if pocketing or layering occurred. Our sampling method minimized disturbance of the lagoon bottoms.

RESULTS

Dissolved oxygen

With the exception of farm H, in Table 1, milking center wastes flowed into the lagoons as generated. Operation H pumped its milking center waste to an uphill lagoon from the barn after three milkings. A time lag occurred because of an arbitrary float setting on the transfer pump used to move settling basin liquids to the treatment lagoon. Both tank odor and liquid color were much like septic tank effluents. A five months lack of dissolved oxygen at the pond might have occurred by the anaerobic predigestion of wastes in the septic tank during intermittent surging.

Farmer W charged his new lagoon with water during a heavy rainstorm. We suspect that much of the water commingled with barnyard manure, thereby exhausting initial amounts of oxygen.

Table 1 shows that dissolved oxygen was always present in three locations, namely J, M, and P. As one might expect, more oxygen was present in upper regions than at bottoms; this was demonstrated colorimetrically by the dissolved oxygen test.

Generally, by increasing the depth of unagitated lagoons, one observes a shifting from aerobic to facultative and ultimately to anaerobic conditions. Sunlight intensity, photosynthesis of algae, and climate conditions influence the kind of biological action in a stabilization pond. Results of our study

TABLE 1. MONITORING OF 6 NEW YORK STATE MILKING CENTER TREATMENT LAGOONS FOR DISSOLVED OXYGEN DURING A 1971-72 PERIOD

| Farms | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | June | July | Aug. |
|-------|--------------------|------|------|----------------|------|------|------|------|-----|------|------|------|
| E | Construction and | | | — ^a | — | — | + | + | + | + | + | + |
| H | prefilling lagoons | | | — | — | — | — | + | + | + | + | + |
| J | with minimal water | | | + ^b | + | + | + | + | + | + | + | + |
| M | amounts. | | | + | + | + | + | + | + | + | + | + |
| P | | | | + | + | + | + | + | + | + | + | + |
| W | | | | — | — | — | + | + | + | + | + | + |

^a— Means no oxygen^b+ Means oxygen was presentTABLE 2. MG/L B.O.D.₅ AT DIFFERENT LEVELS IN MILKING CENTER TREATMENT LAGOONS AT 5 NEW YORK STATE FARMS DURING A 1971-72 PERIOD

| Sampling depth in inches from bottom | Dec. | Jan. | Feb. | Mar. | Apr. | May | June | July |
|--------------------------------------|--------------------------------|------|------|------|-----------------|-----|------|------|
| | <i>(Farm W—Broome County)</i> | | | | | | | |
| 72 | | | | | | | 24 | |
| 45 | | | | 12 | 22 | 24 | 36 | |
| 35 | | | 24 | 12 | 24 | 24 | 36 | |
| 21 | 102 | 36 | 24 | 12 | 18 | 36 | 36 | |
| 9 | 168 | 36 | 30 | 12 | 36 | 60 | 54 | |
| | MEAN | 135 | 36 | 26 | 25 | 38 | 46 | |
| | <i>(Farm J—Wyoming County)</i> | | | | | | | |
| 72 | | | | | | | | |
| 45 | | | | 3 | | | 60 | 18 |
| 33 | | | 12 | 21 | | 24 | 30 | 20 |
| 21 | 120 | 135 | 18 | 24 | 60 | 60 | 30 | 20 |
| 9 | 120 | 21 | 24 | 24 | 58 ^a | 36 | 12 | 36 |
| | MEAN | 120 | 78 | 18 | 59 | 40 | 33 | 23 |
| | <i>(Farm M—Wyoming County)</i> | | | | | | | |
| 45 | | | | | | 12 | 24 | 6 |
| 33 | | | 30 | 30 | 18 | 10 | 6 | 6 |
| 21 | | 15 | 42 | 24 | 24 | 10 | 6 | 6 |
| 9 | 33 | 15 | 48 | 48 | 24 | 10 | 18 | 6 |
| | MEAN | 33 | 15 | 40 | 21 | 10 | 13 | 6 |
| | <i>(Farm M—Cayuga County)</i> | | | | | | | |
| 45 | | | | | 20 | | | |
| 33 | | | 124 | 54 | 20 | 42 | 43 | |
| 21 | | 48 | 36 | Ice | 10 | 42 | 36 | |
| 9 | 220 | 54 | 84 | Ice | 36 | 42 | 42 | |
| | MEAN | 220 | 51 | 81 | 22 | 42 | 42 | |
| | <i>(Farm E—Genesee County)</i> | | | | | | | |
| 45 | | | | | | | | |
| 33 | | | | 104 | 18 | 6 | 6 | 18 |
| 21 | | | 132 | 42 | 30 | 42 | 16 | 10 |
| 9 | | 87 | 44 | 96 | 34 | 24 | 36 | 48 |
| | MEAN | 87 | 88 | 81 | 27 | 24 | 19 | 25 |

^aIce damaged outlet device so as to partially drain pond

suggest that in New York State enough natural mixing takes place through wind and sunlight to cause an agitated pond-like effect. Dissolved oxygen levels in the 6-8 mg/l range were not uncommon and this provides ideal conditions for oxidation lagoons.

Contrary to other studies reported in the literature, we found that the pond's winter ice cap did not prevent aerobic digestion. Figure 1 shows vol-

ume changes which took place during this study with notation of the BOD concentrations then present in the lagoons. Not only was oxygen present in winter, but BOD levels continued to decline. Lagoon odors were absent when dissolved oxygen appeared. A slight "barny" smell was noted in waters without oxygen and only when fluids were held directly to the nose.

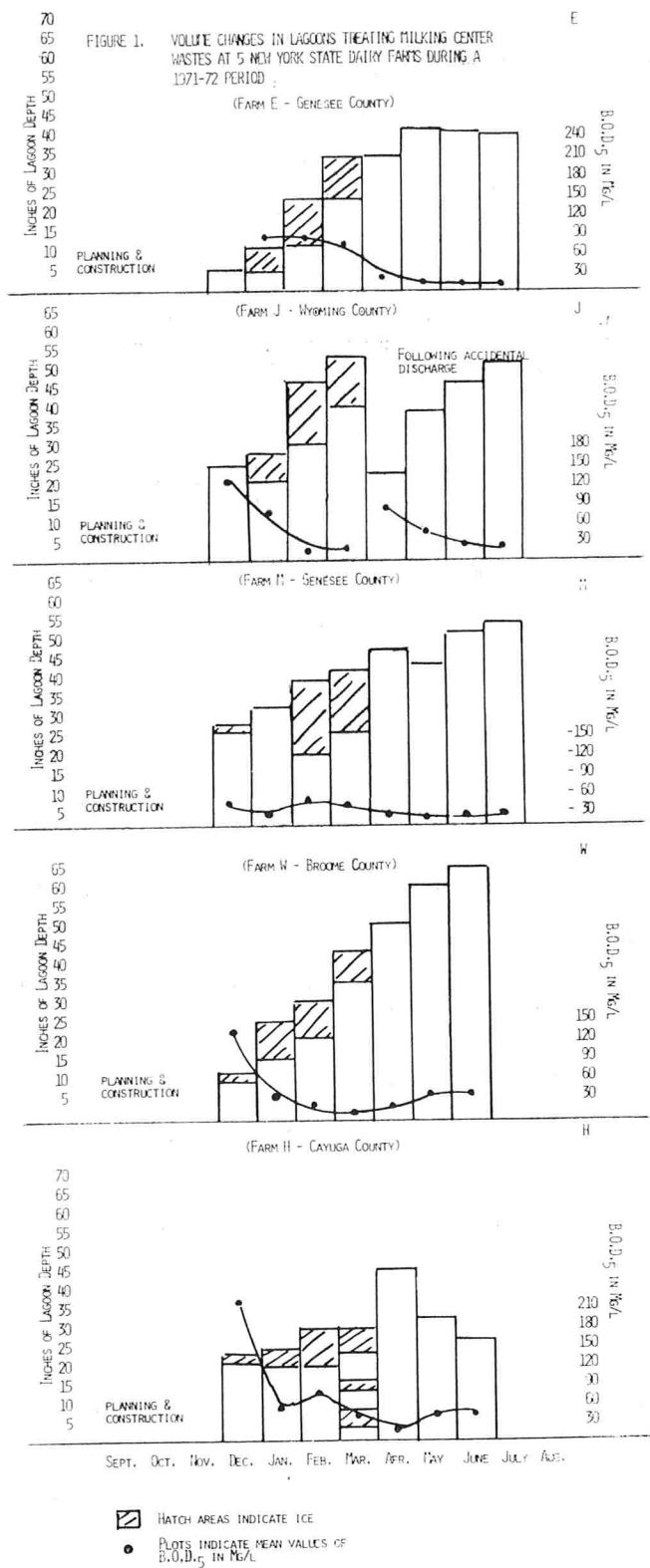


Figure 1. Volume changes in lagoons treating milking center wastes at 5 New York State dairy farms during a 1971-72 period. The bar graph indicates pond depths, hatched areas in graph show ice thicknesses, plot points are mean values of B.O.D.₅ in pond at different depths.

Biochemical oxygen demand

Data in Table 2 show BOD results from five of six locations. Farm P is excluded because the pond's water was diluted with subsurface run-in. A deeply buried field drainage tile was discovered too late for corrective action in early winter.

Initial high organic levels in the pond, at start up, are thought to occur because of water seepage without solids until soils seal. Only minimal differences were apparent at different depths which indicates that waste layering did not occur. In fact, after start up, the data exhibit remarkable operational uniformity. Two accidental milk spills of about 300 lb. each, reported by farmer J in January and February, did not overly shock the lagoon's recovery ability.

Field observations

Part of the study was to document whether or not lagoon treating of milking center wastes cause conditions of nuisance. Our on-site checklist at the lagoons included observations of climatological conditions, pond depth, color, odor, floating debris, algal growth, rodent activity, and others. The "other" category disclosed information such as: (a) When ice appears in the lagoon, a tendency exists for milking center waste to freeze across the pond in layers. (b) Snow accumulations upon a pond's surface vary enormously according to special topography and prevailing wind direction. Farms in the same region capture different snow loads. Farm H, as noted in Fig. 1, had layers of ice top and subsurface. Snow drifted onto the pond and filled its entire depression. Lagoons on farms W and J, on the other hand, were always wind swept clean at sampling visits. (c) Bottom temperature is warmer than liquid temperature below ice cap. The subsurface ground temperature generally raised the water temperature 3-4° F at the 9 inches level as measured by thermocouples attached to the sampling staff. (d) Muskrats, unless controlled at some sites, can damage the side banks of a pond. One animal family apparently made a winter's nest in the discharge pipe on farm M. (e) Frogs were observed in three lagoons as soon as winter passed. The treatment ponds continued to support both fish and frogs at July's end.

Waste volumes and B.O.D.

A previous study of Zall elucidated some characteristics of milking center wastes. Table 3 summarizes pertinent data from that study. Five farms of the group of 24 were randomly selected and re-sampled in April of 1972. Table 4 indicates the findings of this sampling program. Obviously a reduction in hydraulic loading of both volumes and B.O.D. pounds have taken place by different milking center management. Most important to our study is that each repeat farm generated less waste this time than at the June 1971 sampling.

TABLE 3. MILKING CENTER WASTE VOLUMES AND B.O.D. POUNDS—DETERMINED FROM 24 NEW YORK STATE FARMS—JUNE 1971¹

| | Highest individual farm | Lowest individual farm | Average for all farms | Total sampling |
|--|-------------------------|------------------------|-----------------------|----------------|
| Number of cows | 396 | 36 | 100 | 2401 |
| Total gal. of waste generated | 1608 | 111 | 405 | 9713 |
| Total pounds of B.O.D./day | 93.4 | 0.57 | 12.7 | 305.5 |
| Settleable solids ml/l | 200 | trace | 49.5 | 1189 |
| Gallons of waste/cow/day | 16.8 | 1.8 | 4.05 | — |
| Pounds of B.O.D. ₅ /cow/day | 0.38 | 0.012 | 0.127 | — |

Note: (1) Odors from effluent varied from slight feed to strong manure. Colors varied from brown to olive green.
 (2) If the data from the two extreme farms of highest and least number of cows are discarded, the average gallons of waste/cow/day are identical to the 4.05 average with a slight reduction in average pounds of B.O.D.₅ to 0.107/cow/day.

¹Source: Published by R. R. Zall in *Journal of Milk and Food Technology*, January 1972.

TABLE 4. MILKING CENTER WASTE VOLUMES AND B.O.D. POUNDS—DETERMINED FROM 24 NEW YORK STATE FARMS—APRIL 1972

| | Highest individual farm | Lowest individual farm | Average for all farms | Total sampling |
|--|-------------------------|------------------------|-----------------------|----------------|
| Number of cows | 180 | 58 | 104 | 520 |
| Total gal. of waste generated | 440 | 180 | 255 | 1296 |
| Total pounds of B.O.D./day | 4.1 | 2.3 | 3.2 | 15.9 |
| Settleable solids ml/l | 100 | 55 | 89 | 345 |
| Gallons of waste/cow/day | 5.7 | 1.0 | 2.5 | — |
| Pounds of B.O.D. ₅ /cow/day | 0.05 | 0.02 | 0.031 | — |

Note: (1) Odors from effluent varied from slight feed to strong manure. Colors varied from brown to olive green.

Nitrogen, nitrates, and phosphorous

Three lagoons were analyzed for nitrogen and phosphorous content along with total solids. Table 5 shows low concentration levels of elements under test as well as surprisingly little total solids. The liquids were most often crystal clear in appearance. These data indicate that aerobic lagoon treatment has in fact stabilized raw milking center wastes to fluids approaching recreational lake-like quality.

DISCUSSION

Our experience with over 30 new lagoons built in New York State during the 1971-72 period shows that properly designed lagoons acceptably treat milking center wastes. Design standards and specifications for our lagoons were initially prepared by Cornell University and the Soil Conservation Service which set a maximum B.O.D. loading rate of 28 lb./acre/day. Approximately 125 ft² per cow times the number of milking animals have been used as the surface area required at the 2-ft level for designing a treatment lagoon.

If we assume that the present design is oversized for dairy farm milking center loads in New York State as measured by our two field studies, what then might be a better design rule? A panel of educators, conservationists, extension specialists, and industrial representatives visited a sister state in May

1972 to view lagoons treating milking center wastes. Guidelines in that state were established before ours and suggested a sizing of 65 ft²/cow/day as being adequate for treatment. We found that not one pond operated as an aerobic system. On the following day, within but a few miles, the group visited several New York State milking center waste lagoons and found that all operated aerobically.

Candidly, a bulldozer probably can build the big-

TABLE 5. NITROGEN AND PHOSPHOROUS LEVELS IN 3 NEW YORK MILKING CENTER WASTE TREATMENT LAGOONS PRIOR TO BEING USED FOR IRRIGATION. JULY 1972^{1, 2}

| | Micrograms/ml | | |
|-----------------------------------|---------------|--------|--------|
| | Farm E | Farm J | Farm M |
| Total Kjeldahl N | 1.09 | 1.07 | 0.22 |
| NO ₃ & NO ₂ | 0.027 | 0.067 | 0.027 |
| Total phosphorous | 1.68 | 0.68 | 0.77 |
| Total solids | 4.92 | 3.37 | 2.20 |

¹Dr. Keeney, in *The Fate of Nitrogen In Aquatic Ecosystems* at the University of Wisconsin, reports organic nitrogen levels in 12 Wisconsin lakes having monthly mean averages from 0.16 to 0.98 ppm. Lake Winnebago had a range of 0.63-1.91 ppm while at Lake Mendota, amount varied between 0.29-0.85 ppm.

²As of June 1971, 16 states adopted waste water effluent standards. In most cases, limits range from 0.1 to 2.0 mg/l as P, with many established at 1.0 mg/ml.

ger lagoon almost as economically as slightly smaller ponds simply because greater maneuvering care is required in fractional acre areas.

Some engineers, sanitarians, and health officials are requiring two pieces of hardware that together add about one-third to the cost of building lagoons. These structures are settling tanks and out-fall controls. We noted that generally the 1971 construction and equipment costs approximated \$3,500 for 100-cow dairies in New York State. The question is whether or not the benefits from the hardware are measurable. Milking center wastes are principally manure, feed bedding, and hoof dirt which do not readily separate even with centrifugal force. By experience and measurements we can calculate that approximately 1/8 inch of solids will accumulate per year in lagoons sized with current specifications with no tanks.

With basins, it appears that dairymen will have to empty the settling tank about twice a year. It is reasonable to suspect that the soluble organic portion probably passed through septic tanks in the water phase for digestion in the lagoon. Failure to empty a tank filled with solids in time means that the system operates much like a lagoon without a tank except anaerobic organisms seed the pond. Two farms, W and J, of the group did not use settling tanks and their lagoons performed just as well as ponds with settling structures.

If we examine the need for providing outlet structures, one can ponder about their value. Because the lagoon size is calculated to hold waste produced during 400 days, there appears to be little need or advantage to supply large diameter piping to drain the system. In fact, it might be too tempting and easy to drain liquids with outlet structures faster than most land or crops might reasonably accept.

A no-overflow pond with but an emergency spillway is functional for holding milking center wastes. Irrigation devices such as siphon tubes or portable spray pumps can easily withdraw and control the lagoon's liquid to appropriate heights.

The more simple and less costly approach may be to design additional capacity via more surface area to compensate for no-settling tank, if this makes regulatory agents more comfortable.

As we view real world situations, it is obvious that a dairyman can dramatically alter the operation of his waste handling system by poor housekeeping or through careless milk losses.

Lagoons did treat milking center wastes satisfactorily in New York State during this study. Apparently, the treatment pond is not odorous or troublesome, at least, in its first year. Effluents from the system sustain fish and frog life, as characterized by data from Table 5, could be discharged into streams but we do not recommend this approach. It does seem reasonable for farmers to drain the lagoon annually on crops that can benefit from irrigation.

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SERVICE TO HELP MEMBERS ON OSHA BEGUN BY DAIRY & SUPPLIER GROUPS

Washington, D.C., Dec. 11, 1972—"Compliance Information Service," a monthly publication which will contain rules, regulations and interpretations of the Occupational Safety and Health Act, has been established by three major associations representing dairy products and dairy/food supplier companies.

Cooperating in the program, which will be exclusively for their members, are the Dairy and Food Industries Supply Association, the Milk Industry Foundation and the International Association of Ice Cream Manufacturers.

The Occupational Safety and Health Act is the most comprehensive law ever written for the enforcement of safety requirements and healthful working conditions in American business and industry.

Few dairy or equipment and supplier companies have the staff expertise to monitor and analyze for compliance the many regulations, rules, interpretations and the requirements for record keeping under the Act. The Series is designed to provide this much-needed service.

Material will be limited to that which has direct application to the affected industries. Each issue will be printed on sheets suitable for retention in a loose leaf binder, for easy substitution of new materials.

Members of the three cooperating associations will be sent this information at no additional charge as a membership service. Initial issue in the Series is expected in January, 1973.

CLOSTRIDIUM BOTULINUM AND SMOKED FISH PRODUCTION: 1963-1972¹

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ABSTRACT

Occurrence of *Clostridium botulinum* in fish of the Great Lakes was not generally suspected until 1963. Surveillance studies conducted since then have revealed type E to be the most prevalent toxin type in fish and environmental samples of the area. Toxin types A and C, as well as non-proteolytic type B, have been detected only occasionally in Great Lakes fish. Research performed at a variety of laboratories, much of it since the human botulism outbreak traced to smoked fish in 1963, has provided insight into the physiology of *C. botulinum* type E and its spore form. Inoculated pack studies have elucidated conditions of storage which lead to elaboration of toxin. These data have been reviewed and collated with those derived from studies designed to evaluate the Milwaukee Smoked Fish Ordinance. Processing and handling requirements of the ordinance are delineated; the importance of limiting the time and temperature allowed for distribution of this mildly cooked product is emphasized.

Clostridium botulinum type E was first identified by Gunnison, et al. in 1936 (29). Although the original isolate was obtained from a sturgeon in Russia; it was not until some 27 years later that the organism's occurrence in fish indigenous to the Great Lakes became generally suspect.

The first known cases of botulism deriving from fish caught in the Great Lakes were reported in 1960 (5). Vacuum packed, smoked ciscoes were incriminated. The preliminary report stated that mice which had been protected with botulinum A and B antitoxin survived challenge with extracts prepared from leftover scraps of fish. However, a type E strain was subsequently isolated from samples of fish heads, fins, and bones recovered from a garbage can of the afflicted persons 72 hr after the meal (22). Type E toxin was also detected at a level of 100 mouse minimum lethal doses per gram of scraps. *C. botulinum* type E was independently isolated from the fish remnants by at least 2 other laboratories (31). This outbreak aroused the interest of the Food and Drug Administration (F.D.A.) E. M. Foster of the University of Wisconsin was asked by the F.D.A. to conduct a research program directed at determining the prevalence of *C. botulinum* type E in the Great Lakes and its occurrence in smoked fish (13).

Foster's surveillance was begun in June, 1963 ap-

proximately 4 months before the mass media alerted this nation to an outbreak of botulism in Huntsville, Alabama and Nashville, Tennessee. The 1963 outbreak, which has been well described (33), was traced to a shipment of vacuum packed smoked white-fish chubs (6). A later report (7) stated the fish had been caught by gill nets in Lake Michigan on September 16, 1963. They were stored for 1 day at 35 F (1.7 C) before being smoked, allegedly at 180 F (82.2 C), for 5 hr. The smoking temperature was said to have been determined in the air of the smoking chamber. Further testimony suggested the minimum smoking temperature was 175 F (79.1 C). Processing regulations for production of smoked fish in the Great Lakes area did not exist at that time. Consequently, most processors could only guess at the temperature employed during smoking. After having been smoked, the fish were stored at 35 F (1.7 C) for 1 day before being vacuum packaged in clear plastic. Processing and packaging were accomplished in Grand Haven, Michigan. The packaged fish were allegedly packed in dry ice. The shipment left Grand Haven September 19, 1963. It arrived in Nashville September 26. Documentation of refrigeration during shipment was not available. The product was displayed for retail sale September 27. Two human cases of botulism occurred on September 28, after ingestion of fish from this shipment. One of these persons died September 30 and the other October 5, 1963 (33).

Dissemination of this news led many people to suspect that *C. botulinum* type E might be present in fish indigenous to the Great Lakes. A warning was issued by the F.D.A. October 25, 1963. This notice recommended that consumption and distribution of smoked fish taken from the Great Lakes be avoided. A further recommendation was made to the effect that smoked fish should be eaten only if they had been: (a) heated to 180 F (82.2 C) for 30 min after packaging and subsequently kept under refrigeration, or (b) frozen immediately after packaging and maintained in that condition (8, 26).

An account of "tri-level" regulatory control of smoked fish production administered at the municipal, state, and federal level has been published (40). Processing and distribution regulations exercised by the State of Wisconsin and the City of Milwaukee are annotated in that account. The City of Milwau-

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kee ordinance (18) remains in force today as it was adopted in March, 1964. In addition to delineating general environmental and sanitary practices it also prescribes processing and distribution regulations. These include: (a) smoking shall consist of heating every portion of every fish to a minimum temperature of 180 F (82.2 C) for a minimum of 30 min, (b) fish shall be removed from the smoking chamber to a separate room for prompt cooling, and must be packaged within 2 hr after completion of smoking, (c) the packages shall bear the words "Perishable—Keep Refrigerated," (d) the package shall not be sealed so that exchange of air is prevented, (e) the package shall bear the processing date and expiration date, (f) every smoked fish shall be maintained at a refrigeration temperature of no more than 40 F (4.5 C) from the time of packaging, during transportation, and during display, (g) the expiration date for smoked fish shall be not more than 7 calendar days following the date of smoking.

The Milwaukee ordinance was based on data published through 1963. The validity of this decision was affirmed a short time later by K. F. Meyer (37), who stated, "Had the available scientific knowledge, experience and skill of fully trained food technologists been applied between 1960 and 1963, the 12 deaths from commercially processed fish might have been prevented." The Milwaukee Health Department, with financial aid from the United States Public Health Service and the F.D.A. conducted studies to evaluate its ordinance. Some of the data from these studies have been published (41, 42, 43, 44).

OCURRENCE OF *C. botulinum* IN FISH OF THE GREAT LAKES

Outbreaks of botulism cited above and those reported since (6, 9, 10), which also resulted from ingestion of fish caught and processed in the Great Lakes area, left no doubt that *C. botulinum* type E occurs in these fish. However, the frequency and magnitude of contamination among fish and the distribution of contaminated fish within the lakes required elucidation. Data relating to these subjects became available, almost immediately, from the laboratory of E. M. Foster (13). Later reports from the same laboratory (12, 14) revealed that fish caught in Lakes Erie, Superior, Huron, and Michigan contained *C. botulinum* in their intestinal contents in 1%, 1%, 4%, and 9% of the samples, respectively. It is interesting that 56% of 835 fish taken from Green Bay of Lake Michigan harbored *C. botulinum* (12, 55). The wide distribution of *C. botulinum* in fish taken from the Great Lakes, and its prevalence in fish taken from the bays, has been confirmed by Graikowski et al. (27). It appears that the organism readily multiplies in bottom deposits and vegetation of Green Bay (15,

56).

The preponderance of data accumulated from *C. botulinum* surveillance testing of the Great Lakes, and fish taken from them, has revealed the presence mainly of type E organisms. Other toxin types have been encountered only infrequently. *C. botulinum* type A was detected in fish in at least one such study (26). Type C and nonproteolytic type B organisms were detected on 2 occasions, each in another (41). *C. botulinum* type A was also detected in, and isolated from, a whitefish chub in the conduct of thermal destruction experiments described elsewhere (42). This observation has not been published previously. Therefore it will be briefly documented herein. A commercially brined whitefish chub was inoculated with 5.4×10^8 type E spores prior to being heated at 170.6 F (77 C) in an atmosphere of 70% relative humidity (R.H.). Enrichment cultures were prepared from the heated 124.1 g fish in a Most Probable Number series. One culture was comprised of approximately one-half the total weight and 5 cultures, each of 1/10, 1/100, and 1/1000 of the total mass. Only the culture prepared from one-half the ground mass developed toxin. The neutralization pattern observed by mouse inoculation tests is shown in Table 1.

This culture provided further interest as a laboratory curiosity in that only *C. botulinum* type E could be isolated from it upon repeated attempts employing alcohol treatment and a technique previously described (41). Therefore 3 portions, 4.0 ml each, of the enrichment culture were subjected to heat treatment. Each was equilibrated to controlled temperatures in water baths. One was held at 140 F (60 C), one at 158 F (70 C), and the third at 176 F (80 C) for 10 min. Each tube was rapidly cooled in flowing tap water. One milliliter aliquots from each were subcultured to cooked liver meat broth for 7 days of incubation at 82.4 F (28 C). Results of neutralization tests are shown in Table 2. *C. botulinum* type A was later isolated, by alcohol treatment, from the subculture prepared from the portion which had been heated at 176 F (80 C). These data suggest that the type E organism predominated over the type A organism in this particular enrichment culture. It is important to keep in mind that E is not the only *C. botulinum* toxin type which occurs in fish of the Great Lakes.

CONDITIONS OF PROCESSING AND DISTRIBUTION WHICH CONTRIBUTE TO THE BOTULINOGENIC POTENTIAL OF SMOKED WHITEFISH CHUBS

The immediate reaction of many interested persons, following outbreaks of botulism caused by ingestion of smoked fish in 1963, was to assign culpability to vacuum packaging. Although such packaging of

TABLE 1. DETECTION OF TWO TOXIN TYPES OF *C. botulinum* IN ONE WHITEFISH CHUB^a

| | | Neutralization test results | | | | |
|--|-----|---|-------|-------|-------|----------|
| | | 1 × 10 ⁻¹ dilution of enrichment culture | | | | |
| Mice immunized with indicated antitoxin ^b | — | A | B | C | D | E |
| No. mice killed/ no. mice injected | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 |
| | | 5 × 10 ⁻² dilution of enrichment culture | | | | |
| Mice immunized with indicated antitoxin ^b | — | A | B | C | D | E |
| No. mice killed/ no. mice injected | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 |
| | | 2 × 10 ⁻¹ dilution of enrichment culture | | | | |
| Mice immunized with indicated antitoxin ^b | — | A + E | B + E | C + E | D + E | Poly A-E |
| No. mice killed/ no. mice injected | 2/2 | 0/2 | 3/3 | 2/2 | 2/2 | 0/2 |

^a*C. botulinum* type E spores inoculated onto a brined whitefish chub

^b50 French units of each indicated antitoxin/mouse

TABLE 2. SELECTION OF *C. botulinum* TYPE A FROM AN ENRICHMENT CULTURE WHICH CONTAINED BOTH TOXIN TYPE A AND TOXIN TYPE E^a

| | 140 F (60 C) | | | | 158 F (70 C) | | | | 176 F (80 C) | | | |
|--|--------------|-----|-----|-------|--------------|-----|-----|-------|--------------|-----|-----|-------|
| | — | A | E | A + E | — | A | E | A + E | — | A | E | A + E |
| Mice immunized with indicated antitoxin ^b | — | A | E | A + E | — | A | E | A + E | — | A | E | A + E |
| No. mice killed/ no. mice injected | 2/2 | 2/2 | 2/2 | 0/2 | 2/2 | 2/2 | 2/2 | 0/2 | 2/2 | 0/2 | 2/2 | 0/2 |

^aEnrichment culture aliquots heated for 10 min at the indicated temperatures prior to subculture. Broth diluted 10⁻¹ for injection.

^b50 French units of each indicated antitoxin/mouse

smoked fish has not been employed in the Milwaukee area, one processor proposed to install equipment for this purpose. Permission was denied on November 14, 1960. Furthermore, the Commissioner of Health notified all vendors, immediately after the outbreak of 1960, that sale of vacuum-packaged smoked fish would not be permitted in Milwaukee.

It has been known since 1950 (21) and was confirmed in 1956 (38), and later (1, 31, 44) that fish flesh provides an excellent medium for production of toxin by *C. botulinum* type E. Provision of an anaerobic incubation system is not required for the production of toxin in fish. An explanation for this anomaly has been provided by Ando and Katsuhiko (3). These investigators observed that autolysis and putrefaction in fish flesh causes a rapid drop in the oxidation-

reduction potential resulting in sufficient anaerobiosis for growth and toxin production of *C. botulinum* type E.

Thus vacuum packaging is not the only factor which may contribute to the potential for botulinal toxin development in fish. Furthermore, avoidance of this practice, alone, will not ensure production of a safe product. Kautter (31) has reported that vacuum packaging merely extends the apparent shelf life of fish. Visible evidence of microbial spoilage is retarded while production of botulinal toxin is unimpeded. Indeed, the rate of toxin production in vacuum packaged fish is higher than in those sealed in packages without vacuum. The slowest rate has been reported to occur in unsealed packages (1).

The growth of, and toxin production by *C. botu-*

linum type E has been documented to occur within a low temperature range of 38 F (3.3 C) through 50 F (10 C) (21, 39, 52). As would be expected, reproduction time within this temperature range is considerably extended. Nine strains of *C. botulinum* type E were found to yield a mean reproduction rate of 0.0234 divisions per hour at 41 F (5 C) (39). Eklund et al. (23) reported that a heavy inoculum of nonproteolytic *C. botulinum* type B spores produced toxin shortly after gas was detected in 17 days at 42.1 F (5.6 C) and in 24 days at 39.9 F (4.4 C). Growth and toxin production at 39.2 F (4 C) by a type F strain has also been reported (59). Ohye and Scott (39) observed that the minimum temperature at which *C. botulinum* type A and proteolytic type B exhibited growth was 8-10 C above the minimum for type E.

It is clear that maintenance of smoked fish, contaminated with low numbers of *C. botulinum* spores, at 40 F (4.5 C) or below for a limited time should not be hazardous. Licciardella et al. (35) inoculated haddock fillets with a spore mixture of 2 *C. botulinum* type E strains. The fish were irradiated with 200,000 rad of gamma rays before storage at 45 F (7.2 C) or 75 F (23.9 C). After 3 weeks at the lower temperature a *clostridia* count of 1×10^2 per gram and a total aerobe count of 1.9×10^7 per gram of fish were recorded. Sampling was continued at weekly intervals. Toxin was first detected, at a 50% lethal dose (LD_{50}) of 5×10^1 per 0.5 ml, after 6 weeks of storage. Concomitant bacteriologic studies revealed 2×10^6 *clostridia* per gram and 3.2×10^8 aerobes per gram. Toxin was first detected in those fish stored at 75 F (23.9) after 3 days. It also occurred at 5×10^1 LD_{50} per 0.5 ml. The *clostridia* count had increased, by approximately 3 logarithms and the aerobe count by almost 6 logarithms, over the values observed immediately after irradiation.

Inoculated pack studies have been conducted at a number of laboratories. As few as 1×10^2 type E spores have been reported to grow out and produce toxin in vacuum packaged herring in as few as 15 days at 41 F (5 C) (16). Toxin was detected in 11 days under similar conditions in experiments which employed 1×10^6 type E spores per 100 g of fish (57). Vacuum packaged smoked ciscoes inoculated with approximately 1×10^6 viable type E cells were found to contain toxin after only 4 days of storage at 50 F (10 C) (31). Less than 700 type E spores grew out and produced toxin in an open package of smoked whitefish chubs held at 59 F (15 C) for 14 days (44). On the other hand, vacuum packaged herring fillets, inoculated with 1×10^6 spores, contained toxin after storage for only 24 to 48 hr at 68 F (20 C) (1).

It is abundantly clear that the temperature at

which smoked fish are to be held during transit and distribution is critically important. It is equally clear that an inverse relationship exists between time and temperature of storage with respect to their effect on toxin production in contaminated fish.

PROCESSING METHODOLOGY CAPABLE OF MITIGATING THE BOTULINOGENIC POTENTIAL OF SMOKED WHITEFISH CHUBS

An ideal food process would exact each of the following: (a) complete destruction of pathogenic microorganisms and their toxic products, and (b) inhibition of microbial multiplication and elaboration of toxins. The latter objective might be accomplished by chemical additives.

Sodium chloride (NaCl) in a concentration range from 2.5% through 5.8% has been reported to inhibit outgrowth and/or toxin production by *C. botulinum* type E (2, 11, 17, 24, 34, 53). However, inhibition of outgrowth of types A and proteolytic B spores requires a NaCl concentration of 8.2% to 10.5% (34). Unfortunately, a method by which uniform and reproducible levels of NaCl may be attained in Great Lakes chubs is difficult to control (61). It has also been reported that the concentration of NaCl required to inhibit growth and toxin production increases as the numbers of spores present increase (48). Furthermore, the latter study also revealed that low numbers of spores grow out at NaCl concentrations only slightly below the inhibitory level.

Tylosin lactate has been found effective in inhibiting toxin formation in whitefish chubs, inoculated with 800 type E spores after having been smoked at 180 F (82.2 C) for 30 min (54). The inoculated fish were vacuum packaged and tested for toxin after storage for 56 days at 43 F (6.2 C), 28 days at 59 F (15.1 C), 21 days at 65 F (18.5 C), 14 days at 77 F (25.2 C), and 10 days at 86 F (30.2 C). Incorporation of antibiotics into foodstuff, whether intentional or accidental, is not readily sanctioned in this country.

The presence of sodium nitrate ($NaNO_2$) in smoked sable and salmon lox has been suggested as the reason for failure to detect low numbers of type E spores inoculated into them (32). A concentration of $NaNO_2$ at 100 ppm inhibited outgrowth of 4 of 6 strains of *C. botulinum* type E incubated for 180 days at 60.1 F (15.6 C), 50 F (10 C), and 45 F (7.2 C) (24). The rate of uptake and retention of $NaNO_2$ in smoked chub has been investigated by Weckel and Chien (60). It should be pointed out that the role of this salt in the development of carcinogenic compounds, both *in vitro* and *in vivo*, is under investigation (36, 45).

In considering methods by which microorganisms and toxins might be eliminated from foods one pres-

ently has little choice other than the application of heat. The toxins of *C. botulinum* are readily inactivated by heat (35, 46, 47). Spores of toxin types A and proteolytic B are known to be considerably resistant to heat while those of toxin type E are not (30). A general consensus prevails that the latter organism is readily destroyed at 176 F (80 C) (4, 19, 38, 49, 50). Five strains of type E spores, inoculated to a level of 1×10^6 into ground whitefish chubs, were all killed within 17 min at 180 F (82.2 C) (4, 19). The possibility that heat resistant mutants may evolve cannot be overlooked. Dolman and Chang (20) reported survivors among type E spores heated at 212 F (100 C) for 30 min, as early as 1953. Graikowski and Kempe (28) reported survival of type E spores heated at 185 F (85 C) for 120 min and among those heated at 194 F (90 C) for 60 min. It well may be that these observations are a function of spore concentrations used. As was pointed out by Schmidt (51), bacteria are killed at a rate which, in general, is proportional to the numbers present.

Guidelines have been published (58) which recount recommended procedures for commercial dressing of whitefish chubs. These procedures were employed by Fantasia and Duran (25). They reported an occurrence of *C. botulinum* type E in 0.4% of 427 whitefish chubs thus dressed. This value was contrasted to detection of type E organisms among 6.2% of 500 chubs which had been commercially dressed. Application of the dressing guidelines and employment of a germicide, particularly chlorine, as recommended by Ito et al. (30) for in-plant sanitation should be adopted. It does not follow that the requirement for adequate heat processing can be eliminated.

ORDINANCE EVALUATION

Three commercial fish smoking plants in the Milwaukee area have cooperated in studies designed to evaluate the local ordinance (41, 43, 44). *C. botulinum* was found to be most prevalent among whitefish chubs collected from brine tanks in a study in which 66 catches of fish were sampled at 8 stages of processing and distribution. A contamination level of approximately 21% was reduced to about 1.5% among smoked fish which had been heated at 180 F (82.2 C) for 30 min. Corollary data revealed that aerobic and anaerobic populations of bacteria were reduced from several hundred thousand per gram to less than 3×10^2 per gram in fish smoked in compliance with the ordinance. Furthermore, the total bacteria load per gram did not increase significantly in smoked fish held at 33 to 38 F (0.6 to 3.3 C) for 8 days. Smoked fish held at display case temperatures of 39.2 F (4 C), 50 F (10 C), and 60.8 F (16 C) were also examined for aerobic bacteria populations at 8, 16, and 32 days. An increase in storage time from 8

days to 16 and 32 days at 39.2 F (4 C) resulted in a 2- and 3-logarithm increase in aerobic bacteria per gram, respectively. Fish held at 50 F (10 C) for 8 days had populations of aerobic bacteria approximately 5 logarithms greater than those held at 39.2 F (4 C) for 8 days. These data supported the rationale of defining the temperature maximum and shelf life limitation, for holding smoked fish, in the Milwaukee ordinance. The same data have also provided the Milwaukee Health Department with a practical means of monitoring the heating process employed and the retail handling of the product.

In the conduct of surveillance studies, 1,372 samples of smoked whitefish chubs were examined. Twenty one (1.5%) were found to harbor *C. botulinum*. One isolate was identified as a nonproteolytic type B. The remainder were type E. Whether these 21 fish contained spores which had survived the heat process or had become contaminated after processing could not be determined, unequivocally, from the data at hand.

The 3 smoking plants each employed different methods to attain the 180 F (82.2 C) exposure. One, at which a dry smoke process was employed, produced 18 of 896 smoked fish (2%) which harbored *C. botulinum*. Atmospheric moisture of the smoke chamber at a second plant was supplemented by enclosing containers of water. Three of 346 smoked fish (0.9%) examined contained *C. botulinum*. The third processor attained the required temperature exposure by engulfing fish, dry smoked at a lower temperature, with live steam in a chamber designed for this purpose. Only 130 samples of fish thus processed were examined. Not one yielded an enrichment positive for *C. botulinum*.

The low prevalence of *C. botulinum* in fish smoked in compliance with the ordinance became particularly encouraging when contrasted to a survey referred to by Fantasia and Duran (25). They reported detecting *C. botulinum* type E in 14.1% of smoked chubs and in 50% of raw chubs brought into the New York market.

INVESTIGATION OF INTERRELATED PARAMETERS APPLICABLE TO COMMERCIAL SMOKING OF WHITEFISH CHUBS

Review of data accumulated from surveillance of chubs smoked for the Milwaukee market raised an interesting question. It appeared that destruction of type E and nonproteolytic type B spores might be accomplished more efficiently if the fish were heated in an atmosphere supplemented with water vapor. Results of preliminary studies conducted at this laboratory were cited by Angelotti (4). The data indicated that exposure of fish to an internal temperature of 179.6 F (82 C) for 30 min in an at-

mosphere of 70% relative humidity (R.H.) were the minimum requirements for qualitative destruction of type E spores. Conflicting observations were reported by Christiansen et al. (17). These investigators inoculated 1×10^6 type E spores into chubs. Practically all of these fish, which were subsequently smoked at 180 F (82.2 C) for 30 min, produced toxic cultures. Survivors were detected in fish processed in hot air, hot air plus steam, and in steam alone.

Further studies at the Milwaukee Health Department (42) have revealed that quantitative destruction of type E and nonproteolytic type B spores is enhanced by controlling the R.H., during heating of fish, at 60% or greater.

Quantitative destruction of type E spores also was clearly augmented by increasing the exposure temperature from 170.6 F (77 C) to 179.6 F (82 C) and 190.4 F (88 C) in an atmosphere of 70% R.H. (42).

CONCLUSION

Three conditions must prevail for botulism to occur: (a) the spores must be present in the environment and gain access to the food; (b) viable spores must remain in the food after processing; and (c) the food must be exposed to conditions which will allow spore germination and subsequent production of toxin. It is obvious that man can do little to control the first condition. Smoked fish were produced in the Great Lakes region for at least 75 years before the first cases of human botulism resulting from ingestion of these fish were recognized and reported. It follows, therefore, that man must have altered time honored processing and distribution practices about 1960 or shortly before that time. The smoked fish processed in the last 10 to 15 years may have had lower salt concentrations and higher moisture contents than previously. Data to document such speculation are not available. However, there is abundant evidence to prove that, in many geographic regions, packaging and merchandising practices have been changed in significant ways.

The validity of the Milwaukee Ordinance has been sustained by studies we have conducted and by other published research data relevant to the physiology of *C. botulinum*. There are some who may question whether the provisions of the Milwaukee Ordinance are commercially feasible or enforceable. Feasibility is attested to by the fact that several Wisconsin processors have complied with it for more than 8 years. Unfortunately, the shelf life limitation does place some significant restraints on merchandising of the product. It would be ideal if all processors could be convinced of the prudence of complying with the requirements included in the Milwaukee Ordinance. Efforts expended in drafting this review will be well

rewarded if it serves to persuade smoked fish processors to adopt them.

A botulism outbreak which occurs because of indiscretions on the part of a housewife is a tragic incident. One which results from commercially processed food is equally tragic but can also be calamitous to the industry. An industry which has recovered from one such calamity may not survive another.

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LETTER TO THE EDITOR

Five minutes in the diluent still believed to be better than 20 minutes

DEAR SIR:

We noted with interest the recent paper by Hartman and Weber, "Holding times of raw milk dilutions: a reassessment." They are quite correct that the geometric means are a better estimator of changes with time than the arithmetic means we tabulated in the paper they refer to (Huhtanen et al. 1972. "Effect of Time of Holding Dilutions on Counts of Bacteria from Raw Milk," *J. Milk Food Technol.* 35:126-130). The arithmetic means in this paper were erroneous due solely to an oversight on the part of one of us (C.N.H.) and were correctly listed by Hartman and Weber. Our analysis-of-variance of the data, however, was on \log_{10} transforms and perforce was a test of significance of the geometric rather than the arithmetic means. Our geometric means were for 0, 5, 10, 15, and 20 min, respectively: 16988.95, 17900.74, 18538.22, 18631.08, and 18836.32. The percentage changes from 0 time were 5.37, 9.12, 9.66, and 10.87 for the 5- to 20-min holding times. These figures differ from those of Hartman and Weber. The analysis-of-variance showed a highly significant linear trend indicating that the counts steadily increased to 20 min. On this basis we recommended that the dilutions be held no longer than practical (5 min).

Hartman and Weber did not use statistical tests for sig-

nificance of the geometric means or of their "percentage differences" means and used only point estimates for their conclusions. Their "percentage difference" method would have been improved if all changes were calculated from 0 time and an analysis-of-variance done on these differences. Our analysis-of-variance showed highly significant interactions which point estimates would not take into account. We feel that for data such as these, the F test provides a more reliable indicator of change. The F test, however, does not provide a quantitative estimate of the differences. If we accept a total change of around 10% based on geometric means over a 20-min holding time (and agree that we can live with a 10% error), then the 20-min interval between diluting and plating would be satisfactory. We would recommend, however, that if practical, the plating be done within 5 min.

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DESIGN FOR EFFECTIVE MANAGEMENT OF LARGE DAIRY HERDS¹

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ABSTRACT

Few dairies have been adequately designed to meet changing times. Layouts of the 1960's seldom can be efficiently remodeled to meet today's needs. Society's expectations and environmental demands of the 1970's may tend to ineffectuate today's systems. Continuing expansion should be planned for. Most designers plan around a system that starts with cows, adds feed, and produces milk. Maybe they look in the wrong direction for needed results. Manure is the most voluminous product of a dairy; milk the most profitable. What will be their disposition? How many production units (cows) will these outlets allow for? How will these units be operated and managed? What will be the form and availability of raw products, Of nonmaterial inputs? And of management? Products of a large dairy should be the criteria of design for a constrained area. The cow management system that will produce these products can be described, a space allocation made, structures and facilities determined. Finally, the inputs to keep the system functional are considered. The Hi-Eff Dairy is a concept designed around these principles.

Few dairies have been adequately designed to meet changing times. Layouts of the 1960's, usually cast-in-concrete, can seldom be expanded or modified efficiently to keep abreast of continuing herd growth and advancing technology. Now changing social goals and environmental demands have accelerated the urgency for close management.

Most dairy designers have planned around a system that started with cows, added feed, and produced milk. Expressed another way, the dairy was a place to provide for the housing, care, and milking of cows. On the family farm all of these functions were under the supervision of the owner, but might have been the direct responsibility of some other member of the family. As herds grew in size, these separate functions became rather autonomous. In retrospect, the methods used to make each subsystem most efficient did not necessarily optimize the total system.

There are probably few, if any, dairymen, suppliers, or dairy contractors who have thoroughly analyzed (at least for California conditions) the interrelationship of subsystems, structures, equipment, and farming practices which, in concert, make an efficient dairy. For example: one company may provide a

good milking machine; a fabricator elsewhere may supply the finest outside stanchion and feeding equipment; the dairy farm advisor may recommend a bunker silo, while a midwestern manufacturer is bidding on an upright; the general contractor will probably recommend some self-serving design for which the architecture and his methods have been verified. Only the feed dealer may remain silent because he'll sell just as much feed regardless of design. But someone with knowledge of many dairy things and *economics* has to put the package together.

Milk may be the most profitable product of a dairy, but don't forget that manure is most voluminous. What will be the disposition of these two products? How many production units (cows) will developed outlets allow for? How will these production units be operated and managed? What will be the form and availability of raw products? Of nonmaterial inputs? And of management?

The outlets for milk and by-products of a large dairy should be the criteria of design. The cow management system that will fill these outlets can be described, a space allocation made, structures and facilities defined. Finally, delivery of the raw inputs to keep the whole system operating at design capacity are easily scheduled.

WASTE MANAGEMENT

Waste management has become the greatest operating concern of California dairymen. Pelissier, (5) warned of mounting pressure regarding air and water pollution from corral drainage and discharged manure. Dibble (3) has told dairymen the state will be reasonable with agriculture, but he also indicated that dairying should not be viewed differently than other waste dischargers. The International Symposium on Livestock Wastes, April 19-21, 1971, Ohio State University, brought into focus and emphasized the interdisciplinary thrust on this awesome problem resulting from our highly successful livestock production industry. No one now questions that disposal of wastes is more difficult in terms of volume or tons, opportunities or regulations; community acceptance or owner pride; and finally cost; than any other output of dairying. It might appear, then, that waste management should be the logical starting

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point to design a dairy for proper management.

MANURE DISPOSAL

Manure disposal is still best accomplished through land recycling. The soil-plant mantle provides a ready biological system for organic waste decomposition. It makes ecological sense to degrade and then convert nitrogenous waste back into growing plants. Manure can still produce lush pastures if properly managed. Hybrid corn is probably the most effective nitrogen recycling crop because of its wide adaptability, high N requirement, and efficient conversion of NO_3^- to protein—all in addition to its esteemed feed value. Unfortunately, our large dairies which do not have adequate waste receiving land are faced with the costly alternatives of dewatering and exporting waste solids, or of on-site decomposition. Adriano (1) reported alarming soil pollution occurring under dairy waste disposal sites in the Chino Basin. Pratt (6) has indicated an apparent safe recycling capability of only 3 to 5 cows per acre of receiving cropland. The Santa Ana Regional Water Quality Control Board (2) is now regulating dairy waste discharges.

Manure disposal is not a subject of this discussion except as it dictates or influences waste handling procedures up to the point of discharge. But this is the essence of my paper—that the finally accepted method(s) of waste disposal and in what allowable quantities will specify the maximum size and nature of the producing operation. Only after this has been established can the manure handling, cow milking, feeding and shelter systems be quantified. After these components are described we can talk about herd management efficiency.

No dairy should lock itself into a waste disposal plan that either doesn't have back-up systems, or at least can't be simply modified for some alternate method of waste handling. For the foreseeable future I would not limit myself to a liquid or solid system. Each has seasonal advantages and limitations, as well as strong likelihood of a technological advantage under specific circumstances.

HIGH-EFFICIENCY DAIRY

Starting from this background I have developed what I call the HIGH-EFFICIENCY DAIRY. It is a complete drylot layout using contemporary technology for reducing labor, improving production, and simplifying waste handling, while not losing sight of the human limitation of responsibility and the personal goals of ambition and satisfaction (Fig. 1).

Many innovations for housing and caring for cows, for milking, and for reducing pollution have appeared in recent years. Each new idea helped to solve a

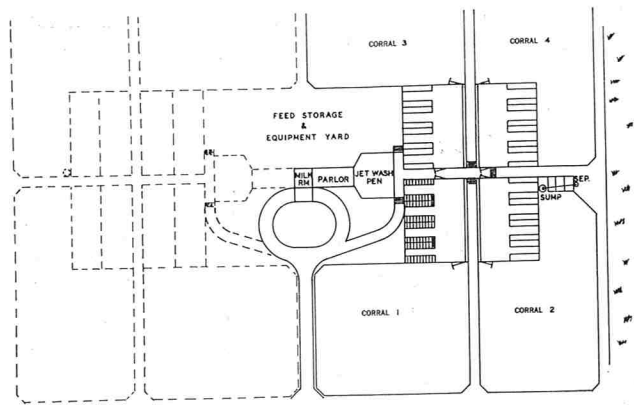


Figure 1. High-Efficiency Dairy.

problem for a dairy or a dairy region by providing relief for some rather specific occurrence or situation. Any one idea might or might not fit into a dairy operation under different climatic, geographic, economic, or social circumstances.

Agricultural engineers and dairy builders have come up with some impressive layouts for herd sizes of about 240 to 2,400 cows. Unfortunately, most of these designs offered little, if any, freedom for the dairyman to try different corral arrangements, feeding methods, loafing conditions, or waste disposal procedures; or for the cow to express or pursue her environmental preference. In general, these concrete milk factories have created only slightly greater envy than rejection by dairymen watching over the fence. Concrete has solved many problems attendant with high density confinement, but also has created some new ones which didn't appear on conventional earth corral, flat barn operations (i.e. foot and leg problems, heat detection, polluted water disposal, inflexibility, and exorbitant costs and depreciation in some instances.)

It has been easy for dairymen to get onto a technological merry-go-round; more difficult to get off. The Hi-Eff design avoids the "all or nothing" limitation of some modern layouts by use of proven concepts, back-up systems, and modular construction.

There have been numerous attempts to mechanize cow handling for work simplification or labor reduction.¹ In most instances familiar to the writer, the labor saving has appeared profitable. However, malfunction does occur and keeps us mindful of Murphy's Law ("If anything can go wrong, it will.") *Mechanization without mechanism* has been the sec-

¹The term "automation" is erroneously used by many dairy suppliers and writers to characterize all forms of powered mechanical systems. An automated system is one with sensing devices to initiate, monitor, and terminate the process. Example: An Albers "Cowwash" is automation; a jet wash pen is mechanization.

ret for success on most outstanding California dairies. The Hi-Eff design would not include a high level of automation initially, but would not prevent its adoption at a later time.

The *Hi-Eff Dairy* is an attempt to integrate our best current thinking about physical facilities and management alternatives consistent with other milk production trends. It is neither a small nor especially large dairy, but is expandable to remain an economical and manageable size for the foreseeable future. (Two or more adjacent Hi-Eff dairies under one-owner management would be logical.)

The *basic precept* of the author is that cows should be kept always close to feed, water, and a protected loafing area so that these factors influencing maximal milk production never become limiting. Travel distance to and from the milking parlor must be minimized to prevent energy waste from excessive walking. Ample exercise area must be provided, but should be on a free-choice basis at the pleasure of the individual cow. All materials-handling to and from the cows—though important in the final design—are not considered in the optimization of the cows' milk production environment.

Size of the basic layout would be a complete dairy of 200 to 400, 240 to 480, 300 to 600, or 400 to 800 cows, but from two strings to eight strings it could be added to in single strings with no loss of efficiency—only a temporary waste of milking parlor capacity until full operation were reached.

Confinement

The *free-stall system* has demonstrated its value in terms of cow population density, space utilization, cleanliness of cows, organization of work routines, centralization of materials to be handled, etc. Free-stall systems have some shortcomings and these will be eluded to in the following discussion. For the Hi-Eff Dairy, then, a compromise to design-in the good points of free-stall housing, and to design-out the known limitations or problems was made.

Our experience favors short (up to 10-cow) rows of free-stalls in order to keep all of the cows close to feed bunks. This arrangement somewhat complicates or restricts manure cleanout, but does provide the more advantageous system for encouraging feed and water consumption (Fig. 2).

Modified loose housing still offers the benefits of management flexibility and minimum structural requirements. The LA RAMS (Los Angeles County Recycled Aerated Manure System) concept of utilizing bio-dried manure as a combination bedding and wet manure blotter may bring back loose housing as a high density confinement system. (There seems to be wide divergence of opinion among dairymen as

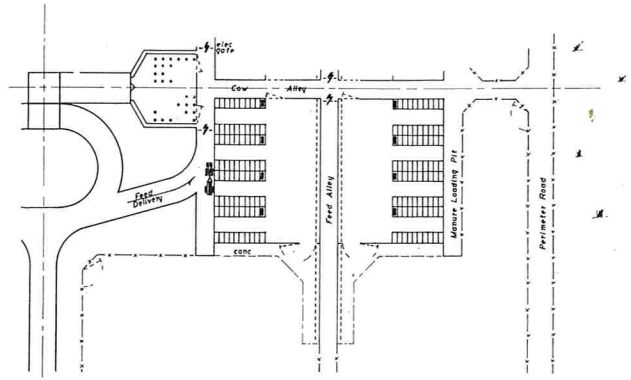


Figure 2. Cow confinement areas with dry manure handling.

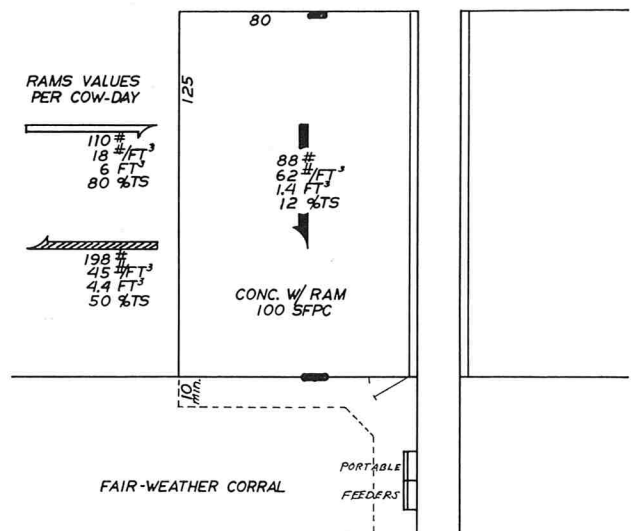


Figure 3. Recycled aerated manure system.

to the teat, udder, and cow injury potentialities of loose housing. It would appear there are some relationships or criteria that were not yet recognized when the free-stall system became popular in the middle sixties.) (Fig. 3).

Either short rows of free-stalls or the loose housing arrangement obviate the controversy of where to place the feed bunks with respect to where the center of mass of cows will be loafing. It is difficult to imagine a more practical way to provide as much dinner table (feed bunk) to as many diners (cows) with unhindered access of waiters (feed trucks) as with the feeding convenience provided in these Hi-Eff arrangements.

High-roofed shelters with all sides open to free-air movement and the plan having nearly square dimensions have proved to be most effective in southern California. This is because shade and heat relief are our most essential needs. A pitched roof creates a flue effect to get rid of warm humid air. A square roof also gives the most rain protection

because of less perimeter for any given number of stalls or loafing pad area.

Alleys would normally be unroofed to get maximum aeration, light, humid air removal, and for the purifying action of sunlight. They could, of course, be totally or partially covered for either rain or sun control if it should be desired.

Optional corrals. Many dairymen do not want to keep cows confined continuously on concrete. Most high density confinement dairies in California have experienced some lameness or other problems that have subsequently been relieved by allowing the cows access to earth corrals or by mounds in the paved areas. The Hi-Eff layout provides about 485 ft² of free-choice, open earth corral per cow. A separate corral is provided for each string and is adjacent to their respective loafing and feeding areas. A 14-ft gate between the loafing area and the corral can be left open or closed at will. The cows would be kept out of the corral during rainy weather to protect the corral surface and to keep the cows cleaner. The corrals are easily entered through perimeter gates for scraping. If experience should show that earth corrals are not economical for the milking herd, they can be used for dry stock or heifers, or can be put into forage production to help recycle the wastes. This would provide the additional benefit of expanding the greenbelt to improve esthetics and to reduce summer temperature.

Pasture is still an economic source of feed and is the most practical disposal crop for recycling of liquid waste. Pasture would fit nicely around a Hi-Eff layout. If the milking parlor were located near the center of the farm, each drylot corral could open onto its own fenced pasture. Gates, lanes, and walking distances would be minimized.

Feed delivery truck drives encircle the dairy core area to simplify the delivery of all feedstuffs. "Hot-wire" drive-through gates, now perfected, would avoid the inconvenience of conventional gates. The feed storage yard behind the barn provides ample turning space for highway transport trucks. There are convenient areas adjacent to the milking barn for employee and visitor parking.

Feeding

Fenceline feeding is attained by splitting the pens and corrals with a double-sided open-ended feed alley. Gates at the ends are not necessary because cows are not moved in this alley. Hot-wire gates are used at the cow alley crossing. This keeps cows out of the feed alley without inconveniencing the feed truck driver.

Two and five-tenths feet of stanchion row per cow is provided so that the entire string can be locked in for observation or treatment. Free-choice feeding

of cubes or other easily-consumed feeds, the outside feeding of concentrates, and the return of small groups of cows from the milking parlor at spaced time intervals would suggest much less manger space per cow if some other opportunity for cow inspection is provided. Presently there is mixed opinion among dairymen on this detail.

Self-unloading feed trucks or wagons have clearly established their superiority in feeding efficiency and flexibility when the roughage can be so handled and the physical arrangement of mangers and alleys permits their use. Chopped hay, cubes, silage, or green-chop can be fed with equal dispatch provided only that the source is convenient. A hay shredder and mixing screw to blend additives would be located in the feed storage yard if baled hay were to be used. The system is ideal for total ration (outside) feeding.

Waste management

Scrape-cleaning of cow alleys maintains a thin manure "carpet" which cushions hoofs and is thought to lessen the occurrence of "concrete lameness". It would seem to have several advantages over the more spectacular flush-cleaning of recent popularity. The benefits of greater cow comfort and a smaller volume of polluted water to manage would tend to offset any slight additional labor. Cow traffic will prevent fly breeding in the alleys. A manure loading ramp alongside each loafing area would be provided if plans were to utilize a nonwetted manure handling system.

Manure-float is the recent European development to facilitate manure removal from stall barns. Basically, the system provides a grated gutter or trench approximately 75 cm wide (30 inches) and up to 2 1/2 m deep (8 ft) immediately behind the stalls. It runs the length of the barn and leads to a sump outside. The trench is dammed with a sluice gate adjacent to the sump and it is nearly filled initially with water. The water is to maintain a slurried condition and prevent the drying and lumping of solids. The urine and manure are accumulated for up to 30 days. When the gate is raised, the slurry flows slowly into the sump and is later spread onto cropland.

A *combined system* of flowing reclaimed or recirculated water through a deep trench, as just described, would seem practical for our California conditions. The sluice gate might be eliminated if experience shows that manure encrusting on the sides of the trench does not cause a problem such as fly breeding, extra odor, or damming. A combined flow system would move some portion, if not all, of the waste daily into the sump. This would be more in keeping with our continuous flow schemes. If the sluice gate were installed, however, the trench could

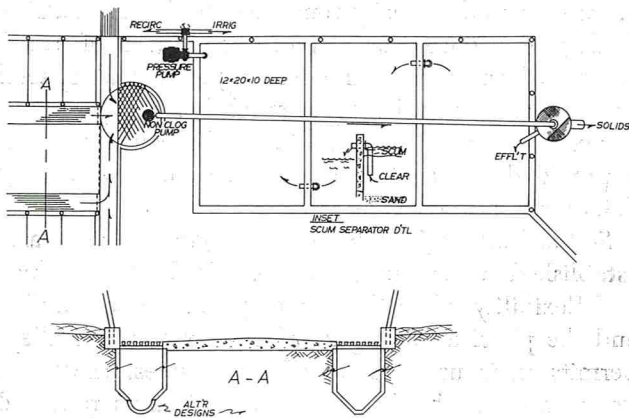


Figure 4. Liquid flush cleaning system with solids separation.

store a month's accumulation of waste if irrigation and fertilization schedules suggested it (Fig. 4).

There would be no drying of the waste in the trench, but there would, of course, be some odor generation. There need not be any long hay feeding in the Hi-Eff Dairy, therefore, no problem with fouling or plugging of trenches, pumps, and other equipment.

Tractor scraping of alleys and slabs would probably be a daily to twice-weekly chore. (As mentioned above, a semi-dry manure layer should provide a better walking surface than washed concrete.) All-grated gutters would make tractor scraping a relatively fast chore. At least one-half of the total manure would fall directly into a gutter and more would be worked in by the normal movements of the cows. Each pen could be scraped while that string is being milked. A constant small flow of wash water from the third settling tank would likely be sufficient to keep the manure flowing.

A *round sump* catches all of the waste and an open runner or shredder pump lifts the slurry up to a separator device. The fiber solids are removed for free-stall bedding, for bio-drying (Recycled Aerated Manure System) or for plant mulch; the effluent is caught in a settling tank.

A *three-tank system* clarifies the effluent for recirculation by settling and skimming. Cow and

parlor washing would provide make-up water. An amount of effluent at least equal to the make-up water is diverted to an irrigation reservoir, aeration pond, or treatment system. Continuous aeration of the reservoirs may be necessary in some localities to control anaerobic odors.

As research develops less costly methods of reducing the energy level, recycling, or decomposing organic wastes, the Hi-Eff Dairy will be ready because of its engineered waste collection system.

CONCLUSION

Any modern dairy represents a capital investment well over one-thousand dollars per cow. A dairyman considering a new facility should examine his and his heir apparent's 20-year plans before making so heavy a commitment.

I believe technicians and engineers, working with all segments of the industry, are qualified to put it all together. They have close access to the best information on feeding, cow care, and milking; also the essentials of successful farming and environmental protection—all without being beholden to profit. Working with dairymen and their suppliers they can infuse the principles of materials handling without losing sight of biological or animal factors. A large dairy that is not well planned cannot be effectively managed.

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A Research Note

RELEASE OF ENTEROTOXIN FROM WASHED CELLS OF STAPHYLOCOCCUS AUREUS¹WILLIAM C. HAINES² AND L. G. HARMONDepartment of Food Science and Human Nutrition
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ABSTRACT

Enterotoxin was detected in lysates from washed cells of *Staphylococcus aureus* treated with toluene, lysozyme, and acetone-dry powder; but no toxin was detected in lysates from washed cells disrupted by sonication, grinding, or freezing and thawing.

Various workers have attempted to demonstrate intracellular staphylococcal enterotoxin by disrupting cells, generally without success. Previous reports (3, 6) stated that no significant intracellular toxin could be obtained from cells of *Staphylococcus aureus* in various stages of growth. Recently, however, Baird-Parker (1) indicated that treatment of washed cells of *S. aureus* with lysostaphin can effect a release of small quantities of enterotoxin. Thus a variety of cell disruption techniques were applied to washed cells of *S. aureus* 243 (ATCC 14458) harvested from the stationary phase of growth after incubation for 24 hr at 37 C.

MATERIALS AND METHODS

Cells were grown in Brain Heart Infusion broth incubated at 37 C under constant agitation, harvested by centrifugation at $7900 \times g$ for 30 min at ambient temperature and washed twice with a volume of physiological saline equal to the original volume of broth. A third washing was done with a volume of saline 1/50 of the original broth volume. The supernatant from the original culture and the washings were assayed for enterotoxin B by the procedure of Casman and Bennett (2). Washed, packed cells were disrupted as described by Vadehra et al. (7). In addition, a 4-g portion of the cells was brought to a volume of 20 ml with de-ionized water, mixed with 0.4 mg of lysozyme (Nutritional Biochemicals Corp.), incubated at 30 C for 2 hr, centrifuged at $12,000 \times g$ for 30 min and the supernatant was recovered. All lysates were assayed for enterotoxin. In addition, the absorbancy at 280 nm (A₂₈₀) for each of the lysates diluted 1/20 with de-ionized water, was determined on a Beckman DBG spectrophotometer to estimate the amount of total protein released.

RESULTS AND DISCUSSION

No enterotoxin was detected in the lysates produced by sonic treatment, grinding, or freezing and

TABLE 1. COMPARISON OF THE ABILITY OF SEVERAL DISINTEGRATION PROCEDURES TO RELEASE ENTEROTOXIN B FROM WASHED CELLS AND ABSORBANCY AT 280 NM OF 1/20 DILUTIONS OF CELL EXTRACT SOLUTIONS OBTAINED FROM DISINTEGRATION OF CELLS OF *S. aureus* 243 BY THE PROCEDURES INDICATED.

| Disintegration procedure | Enterotoxin B ($\mu\text{g/ml}$) | Protein released by disruption (A ₂₈₀) |
|--------------------------|------------------------------------|--|
| Grinding | N.D. ^a | 0.48 |
| Freeze-thaw | N.D. | 0.60 |
| Sonication | N.D. | 0.61 |
| Acetone-powder | 1 | 0.65 |
| Toluene | 1 | 0.88 |
| Lysozyme | 1 | 0.43 |

^aNone detected.

thawing (Table 1). Toluene treatment, lysozyme treatment, and the acetone-dry powder procedure effected the release of a quantity of enterotoxin B which could be detected. No enterotoxin was detected in any of the second and third rinse solutions.

Known crude enterotoxin preparations were subjected to sonic treatment, grinding, and freezing and thawing to determine if these procedures denatured the enterotoxin in a manner to render it non-detectable by the double-gel-diffusion procedure. No denaturation was observed by any of these procedures. It must, therefore, be concluded that no detectable quantity of toxin was released from the washed cells by these procedures.

There was no consistent relationship between ability to release enterotoxin and total protein released as determined by the A₂₈₀ of the lysates (Table 1). Procedures which resulted in detectable quantities of enterotoxin B in the lysates apparently had a degree of specificity for enterotoxin sites. Procedures which were successful are those which are generally most effective against the cell surface. Previously, Friedman and White (5) demonstrated enterotoxin on the cell surface by a fluorescent-antibody procedure. This toxin is bound weakly to the outside surface of the cell wall and is removed by conventional washing procedure. In addition, Friedman (4) reported inhibition of synthesis of enterotoxin B by agents which block the synthesis of the cell wall. Results obtained here also tend to indicate enterotoxin is associated with components of the cellular surface of *S. aureus*.

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BOOKLET ON FOODSERVICE FILMSTRIP TRAINING

A new booklet, "Profits and Your People—Through Foodservice Filmstrip Training", has been published by the National Restaurant Association. This booklet contains a trainer's guide for each NRA sound-filmstrip training program. It also contains achievement tests for every sound-filmstrip, each test prepared for local reproduction and use.

Included in the kit is a sample of a recommended NRA Achievement Certificate to be awarded to employees completing training. It has been designed for use in restaurants and other food-service op-

erations, in public health department programs, and in foodservice education courses for adults and for students at both the secondary and post-secondary levels.

Single copies of this publication can be purchased for \$2 from the Educational Materials Center, National Restaurant Association, 1530 N. Lake Shore Drive, Chicago, Illinois 60610. NRA sound-filmstrip programs referred to in the publication also can be purchased from the Educational Materials Center.

AGRICULTURAL SANITATION OF LIVESTOCK MANURES FOR CONTROL OF FLIES, ODORS, AND DUSTS¹

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ABSTRACT

Industrial agriculture and urban-rural growth are mainly responsible for the FOD (Flies, Odors, Dusts) nuisance problems associated with livestock industries in many states. Manure from animal confinement type operations is a major source of these nuisances. Drylot dairies, beef feedlots, and raised wire-cage poultry ranches are the main industries which affect the co-existence of agricultural and suburban living. An Agricultural Sanitation Program by the University of California consists of these principal activities: research, demonstrations, and education. Physical, mechanical, biological and chemical methods are combined into an integrated control program with major emphasis on manure management including collection, disposal, and use. Cooperative research programs also are made with interdisciplinary personnel representing federal, state, and local agencies in line with state and local codes and ordinances governing control of FOD problems.

The co-existence of agriculture and suburbia is a problem which demands control of the FOD (Flies, Odors, and Dusts) nuisance problems, particularly those associated with livestock industries. Two factors, industrialized agriculture and urban growth, have proceeded so rapidly that head-on confrontations are the rule in many states, and specifically in California where the human population has changed from sharing 1,000 acres to only 5 acres per individual. Prime agricultural land has gradually been black-topped to accommodate the growth of urban and industrial developments. This encroachment upon farm land, however, has not deterred the farmer in keeping pace with a greater demand for food production. On the contrary, today's agriculturist can produce far more per acre than before and in some instances, he is subsidized not to overproduce. Equally so, consumer demands have required greater production of a wide variety of food types along with special preparation and packaging in order to help satisfy a prosperous and affluent society; excessive waste is a by-product of these production and marketing efforts and constitutes a considerable part of the retail price of food. At the same time, we now demand greater control of the FOD problems, but the satisfactory financing of abatement methods is yet

¹Presented at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, San Diego, California, August 15-19, 1971.

to be determined.

Here then, is the core of the problem, wastes from living and wastes from making a living, both from the producer and consumer attempting to live together within smaller land units. When these same land units are occupied by animals, the problem is further complicated. Our sanitation engineers tell us that nationally, we produce 100 gal of sewage per capita per day resulting in approximately 185 million yd³ of solid wastes per year which, to a large extent, is managed and disposed of by standard municipal sewage treatment plants whose operational costs are financed in part by the homeowner. Our agricultural engineers tell us that just with farm animals alone the manure production in the United States is about 1 billion yd³ per year. This volume of manure is managed and disposed of by a wide variety of methods, and in general, is financed on an individual animal-owner basis. As a wide interest, one can add to these staggering amounts, the 4 million tons of manure from 50-plus million dogs which have access to backyards, streets, parks, and beaches each year.

Because of these factors and the need to control FOD problems of public health concern, the University of California, numerous State Colleges, and the State Departments of Agriculture and Public Health placed more emphasis in the early 1950's on separate but coordinated programs of basic and applied research as well as on revision of state and local codes and ordinances to benefit community management of solid wastes. Included in solid wastes was the management and use of animal manures from close to 153 million livestock and poultry under confinement-type operations (Table 1). The Agricultural

TABLE 1. MANURE PRODUCTION NEEDING REMOVAL FROM LIVESTOCK OPERATIONS¹, CALIFORNIA, 1969.

| Type | Livestock | | Manure production in tons | |
|---------|-----------------|--------------|---------------------------|--|
| | No. in thousand | No. produced | Air dry (90%) | |
| Dairy | 1,550 | 18,700,000 | 3,700,000 | |
| Beef | 882 | 2,700,000 | 738,000 | |
| Poultry | 151,300 | 2,181,000 | 808,000 | |
| Swine | 86 | 1,200,000 | 27,000 | |
| Horses | 120 | 427,000 | 154,000 | |

¹Animals under confinement.

Research Service of the U. S. Department of Agriculture also contributed to field studies in waste management by assignment of interdisciplinary personnel at specific problem-area field stations; agriculturists, engineers, and entomologists cooperated with similar personnel in state and local agencies as well as those working in the University and State College systems.

An outgrowth in 1960 within the University of California research programs was establishment of an Agricultural Sanitation program supported by specialist staff in the Agricultural Experiment Station and the Agricultural Extension Service. The program was divided into three main activities: research, demonstrations, and education. Program elements were not the sole responsibility of any one group but the joint responsibility of interrelated disciplines representing agronomy, animal and poultry sciences, economics, entomology, engineering, irrigation, range improvement, and soils.

Similar lines of attack have occurred around the country and educational institutions and governmental agencies are active in such states as Florida, Illinois, Iowa, Michigan, Minnesota, Nebraska, New York, Ohio, Pennsylvania, Texas, and Washington. Representative of the work effort on this subject was a series of initial meetings beginning with the "National Symposium on Poultry Wastes" and "What's New in Farm Manure Disposal," 1963, as well as "Agricultural Wastes Management," and a "National Conference on Solid Waste Research," 1964. An outgrowth of these early conferences stimulated international participation in symposia on livestock wastes, the latest meeting being held, at Ohio State University, April, 1971.

Results from nationwide studies on basic and field research have shown that there are answers for the control of FOD problems. In many instances the solutions are simple but implementation of these solutions is difficult. Experience has shown that there

is no one method to solve all problems and complications arise because each problem has to be considered individually. This then leads to repetition of the statement that one man's stench is another man's perfume; that people are paradoxical and that people problems are the real issue. The latter factor often escapes consideration and study.

DOMESTIC FLY CONTROL

Although as many as 50 or more different fly species may be attracted to or originate from livestock operations, relatively few of these species occur in large numbers or have noxious habits to make them serious pests of man or livestock. Conversely, however, the few troublesome species of "filth" or domestic flies make up for their scarcity in species by their high rate of reproduction, their ability to disperse many miles from a breeding source, their pestiferous action, and their ability to transmit disease agents to man and animals. A list of the more important domestic flies associated with livestock operations is shown in Table 2 (10). We must bear in mind that these flies are intimately related to man and by his management of livestock in placing more animal units per square foot of confinement. Drylot dairies, beef feedlots, raised, wire-cage poultry ranches, and multiple-pen indoor houses for swine are all examples. Collection, processing, and disposal of manure from these operations can be managed by various methods so as to avoid production of flies. These manure management methods, therefore, are well understood but overall producer acceptance has not been achieved because of added production costs. For example, it has been estimated that treatment of drylot dairy waste in the same manner as city sewage would require a retail price increase of about 4 cents per quart of milk. Is the consumer ready to pay this portion of an overall greater expense as one part of a nuisance-free environment?

TABLE 2. DOMESTIC FLIES COMMONLY ASSOCIATED WITH ANIMAL MANURES IN CALIFORNIA.

| Popular and scientific names | Number days for life cycle (Egg to adult) ¹ | Animal manures recorded from: | | | | |
|---|--|-------------------------------|-------|-----|-------|---------|
| | | Cow | Steer | Hog | Horse | Poultry |
| Black blow fly, <i>Phormia regina</i> | 10 | | X | | | X |
| Black garbage fly, <i>Ophyra</i> species | 10 | X | | X | | X |
| Blue blow fly, Calliphoridae (species) | 15 | X | | X | | X |
| "Coastal fly", <i>Fannia femoralis</i> | 14 | | | | | X |
| Green blow fly, <i>Phaenicia</i> species | 8 | X | | X | | X |
| False stable fly, <i>Muscina</i> species | 14 | X | X | X | X | X |
| Flesh fly, Sarcophagidae (species) | 8 | X | X | X | | X |
| House fly, <i>Musca domestica</i> | 8 | X | X | X | X | X |
| Little house fly, <i>Fannia canicularis</i> | 24 | X | | X | | X |
| Stable fly, <i>Stomoxys calcitrans</i> | 21 | X | X | X | X | X |
| Drone fly, <i>Eristalis</i> species | 21 | X | X | X | | X |

¹Relative time during warm summer temperatures. Cooler or very hot temperatures will, respectively, retard or accelerate this developmental period.

TABLE 3. CHARACTERISTICS OF RECOMMENDED INSECTICIDES FOR CONTROL OF ADULT FLIES, CALIFORNIA, 1971.

| Insecticide | Toxicity ¹ to man | Rate of fly knockdown | Residual activity ² |
|----------------------|---------------------------------|--------------------------|---------------------------------|
| Ciodrin | moderate | slow | 1-3 weeks |
| Diazinon | moderate | slow | 4-6 weeks |
| Dimethoate (Cygon) | moderate | slow | 2-6 weeks |
| Dichlorvos (Vapona) | high | fast | short (in strip form, 3 months) |
| Dylox (Dipterex) | low | slow | very short (bait) |
| Fenthion (Baytex) | moderate | slow | 2-4 weeks |
| Malathion | low | slow | 1-2 weeks |
| Naled (Dibrom) | low | fast | 1 week (longer if cool weather) |
| Pyrethrins-Pyrethrum | low | fast | none |
| Rabon | low | slow | 2-3 weeks |
| Ronnel (Korlan) | low | slow | 2-4 weeks |

¹All insecticides listed are poisons.

²Activity varies from area to area or ranch to ranch depending upon type of surface sprayed, dosage used, weather, and susceptibility of flies to chemical.

Historically, solutions to fly control have long been established by barnyard methods of manure management and of manure use as a fertilizer. With the change to greater use of agricultural mechanization concurrent with the introduction of DDT and similar type insecticides, fundamentals of fly control were largely forgotten.

Control methods, therefore, have been largely temporary through use of insecticides applied either to farm structures as adulticides or to livestock manures as larvicides. A list of those insecticides recommended for use in California and undoubtedly duplicated for use in other states is shown in Table 3. These insecticides, if properly chosen and carefully applied, are extremely useful and effective for fly control. The value of insecticide treatment, however, should be judged by the extent of reduction in the number of living flies and not by the number of dead flies found on the premises. Likewise, entomologists have strongly recommended that insecticides should be used at the early part of the summer season when flies first begin to be observed (1, 6). This is contrary to the idea by many livestock operators who always wait to use insecticides at a time when their premises are under attack by dense numbers of flies.

The need for frequent use of these chemicals has resulted in resistance to insecticides by numerous fly species. The pattern of insecticide resistance by flies across the nation, as well as in some areas of Canada, has reached the point where we must depend upon chemicals which are highly toxic to man and animals in order to satisfactorily control many pest species (4, 7, 8). Continuous larviciding of animal manures, in particular, only accelerates appearance of chemical resistance by flies. Also, insecticide applications to manures cause high mortality of beneficial arthropods—those which help degrade the substrate and

those which act as predators and parasites of fly eggs, larvae, and pupae. Although some of these beneficial arthropods have long been recognized, it is only within the last 10 years that scientists have determined their real value and have been able to successfully cultivate them inside the laboratory in sufficient quantity for release as biological control agents (3, 9). Of recent encouragement is the commercial production of wasp parasites for control of domestic flies found breeding in poultry manure. The Vitova Company in California is now expanding its investigations to include use of similar agents in dairy manure. The present role in the use of insecticides is incorporated into what is called "Integrated Control." Results from field studies in California (2, 4) and in North Carolina (3) have demonstrated excellent domestic fly control on poultry ranches by a program of manure management and restrictive use of insecticide applications. Results from one of the California studies (5) showed better fly control from maximum efforts in systematic mechanical collection and rapid drying of poultry manure; costs of frequent manure clean-out and management ranged from 4.2-13.8 cents per bird per year with hand labor influencing the higher cost and not necessarily doing a better job.

Application of integrated control practices for dairy cow manure is not new as the trend towards drylot operations has required consideration of rapid management of a greater concentrated volume of cow manure. Weekly to bi-weekly collection and removal were a necessary procedure but were particularly difficult for small dairies. The annual cost for an 80-cow "cash and carry" dairy amounted to \$886 for weekly manure collection, loading, and spreading. Although no FOD problems were produced, the dairyman still had to spend \$300 per year for insecticides to control flies originating from elsewhere in the community. The

total expenditure of nearly \$15/cow per year is considered a normal cost for dairy operations of this type. The disposal factor, or ultimate use as a fertilizer, has been a bottleneck in some regions. For example, the present 20 million people in California are mainly distributed in three major metropolitan areas: southern (chiefly Los Angeles), central coastal (San Francisco Bay Area), and the Central Valley. Peculiarly, this division of human growth parallels the growth of the dairy industry: 45% in the Central Valley, 30% in southern Los Angeles area, and 15% in the central San Francisco Bay area. Thus, the anticipated growth in California population to about 24 million in 1975 with only a small decrease predicted in the number of dairy cows, requires new concepts of planned sanitation to enable coexistence of man and animals in these three areas. The California picture is merely a reflection of similar problem areas experienced in some of our other states. If we can minimize production of flies so as to create a satisfactory living level for man and animals, then we should be able to achieve the same level regarding dusts and odors. It should be remembered, however, that in the discussion concerned with fly control, it was obvious that the reduction of insecticide use relied heavily upon the correct management of animal manures. In so doing the interaction of the OD (odors and dusts) problems can be innocently activated to a problem level.

DUST AND ODOR CONTROL

Poultry operations

Dust problems originating from livestock operations may be similar in some respects but different in others. For example, dust problems used to be a major problem on turkey ranches. The problem was not one concerned with manure but rather with the fine-particled size dirt layer common to those areas of little if any rainfall from spring through fall. Turkey growers tried many soil treatment methods such as sprinkling various oil products, application of wood shavings, or running a water-sprinkler truck over the area, to name a few. A well designed sprinkler system which operated on a regular schedule was ultimately found to not only control dust but it also improved bird growth and egg production. Disadvantages, as commonly encountered with almost all systems, arose from poor maintenance of sprinkler heads and in the over use of water. Also, excessive ponding and mud holes contributed to production of odors and to increase in transmission of infectious disease.

While dealing with poultry, it may be appropriate to include the problems of odors and feathers originat-

ing from layer operations. Despite the early attempts to solve these problems by the use of chemical deodorants or burning, the overall problem has been minimized by: (a) frequent manure collection and off-ranch removal and (b) poultry house enclosure. Either method has required a large capital investment in manure handling equipment or in building construction, or both. Satisfactory control of these nuisances along with minimal fly production has increased the incentive to produce a manure stockpile more suitable for fertilizer application.

Dairy operations

Frequent manure removal by physical methods has been interpreted in the age old phrase "wet it or dry it." Complete wetting of manures by a liquid system is more common on dairies where milk barn manure and wash water are emptied into manure ponds or sumps, ditches, or directly into irrigation pipes for surface or overhead application to grazing pastures or to crops. In any event, the effluent can and should be used for irrigation and fertilizer value if adjacent cropland is available. However, such liquid systems on dairies only account for about 10-40% of the total daily manure deposited by cows. The danger of the manure ponds is the factor of overload of solids resulting in anaerobic digestion and production of offensive odors. With flush clean dairies (100% concrete) or on other dairy operations which turn to drylot management, there is usually less acreage for use of a liquid fertilizer system. On dirt-corral drylot dairies, handling of manure must be made on a frequent basis throughout the year and manure fertilizer plants operating within a dairy-industrial city have been the tentative answer until real estate values exceeded the return from a glass of milk. Some dairies engaged in a dry system of manure management have used deodorant chemicals during the time of corral cleanout in order to minimize the offensive smell which results from disturbing layers of manure undergoing anaerobic digestion. This results in an additional expenditure of 50 cents to \$1.00 per cow per year which in nearly all instances is not returned to the dairyman from the sale of bulk manure.

Feedlot operations

One of the biggest problems with regard to odors and dusts is that associated with beef feedlot operations. Here the FOD problem reverses itself to be known as "DOF" in descending order of priority nuisance problems.

Investigations aimed at finding both short and long-term solutions are concentrated in the four main cattle feeding regions (16): the northern area in line from North Dakota through Nebraska to Colorado, the Corn Belt area, the panhandle area of Texas and

Oklahoma, and the southern dry desert areas of Arizona and California.

Dust clouds begin to hover over feedlots in the early evening hours during the warm summer months as a result of the activity of thousands of cattle. Along with the evening temperature conversions and the increase of wind currents, these dust clouds begin to disperse from the premises and if moved in the direction of the nearest town, the results are quite obvious. Traffic flow has often stopped along highways bordering feedlots because of the dense formation of dust clouds. Equally disturbing, particularly to newly established inhabitants in the environs, is the wide array of odors which come and go throughout each 24 hr period. Even the smell of "green chop" used in feedlot rations has been responsible for public complaints.

The dust problem has been reduced on a local basis first for the comfort and health of the cattle, secondly for feedlot personnel, and thirdly for the inhabitants living in the nearby town. Water-tank trucks have given way to construction of an overhead sprinkler system which operates prior to and during periods of animal activity. A dust control system of this type for a 30,000 head-capacity feedlot would normally cost about \$30,000 or one dollar per head capital investment. The system, in this instance, included a 250,000 gal reservoir, a 1000 gpm pump, a 60 HP electrical motor, 600 sprinklers, and about 2 miles of main and lateral pipe lines. But pen size and shape very often determine the type of system and equipment for use. For example, deep pens cannot be adequately covered from tank trucks and overhead shade structures within pens may interfere with a sprinkler system. As with any water system, poor maintenance of sprinkler heads or the overuse of water from tank trucks can result in an increase of odors and also the excess wetting of manure which then increases fly production.

With those feedlot operations which use maximum animal crowding per pen space, dusts have been eliminated without the occurrence of the secondary problem of odors. Results from field studies on feedlots in hot and arid areas of southern California have shown that from 60-100 ft² per animal allowed satisfactory weight gains concurrent with control of dusts and odors (11, 13). Other feedlot operations may choose to conduct a routine monthly cleanout and disposal system. In some instances, prior to the time of pen cleanout, manure surfaces are sprayed with various soil binders such as calcium sulfate, or with various deodorants.

Even under most ideal conditions, however, deodorants are really masking chemicals and in no way are formulated to counteract the numerous chemical

compounds in manures that come from highly variable rations fed to different age cattle within the same feedlot. The end product which undergoes putrefaction on the surface of the feedlot pen is also subject to extremes in environmental conditions. It is all the more difficult to identify odors other than what the human olfactory sense usually distinguishes as urea and sulfur compounds; unidentified odors may include numerous volatile amines and organic acids. The most difficult tasks are collecting samples of ambient air and the determination in parts per billion.

Preliminary results from initial studies on identification of certain odors from California feedlots showed that trimethylamine was the most odorous of all amines tested by gas chromatography (14). It was readily admitted, however, that field sampling devices still need to be perfected in order to trap odorants arising from many different environmental conditions and that laboratory detection techniques should include use of liquid chromatography, infrared spectroscopy, or flame photometry, to name a few.

Factors listed for successful feedlot operations in northwestern states are simple but again, are difficult to practice in consideration of the economics of management; factors include location of operations away from people, avoidance of spilling wastes on the land or into potable waters and the provision of adequate cropland for manure disposal (12). In reality, these factors apply to almost any high density, animal confinement operations whether for cattle, cows, horses, poultry, sheep or swine.

Management of confined animals

It is little wonder, therefore, that confinement-type animal management systems have been tested and in some instances placed in operation under enclosed housing. "Out of sight, out of mind" may apply here with respect to recognition of the basic "people problem." For example, the completely enclosed poultry house can be made to blend into the surrounding environment so that the public cannot see or hear poultry activities with the result that there is less chance of public complaints. Complete nuisance control of flies, dusts, odors, noise, and merely the sight of domestic animals can be achieved by enclosed housing as long as animal health and production are maintained. Enclosed systems which include light, temperature, and humidity control for poultry or swine management have become more common in certain critical suburban areas. Hog feeders and poultrymen have found that the FOD problems can be controlled from within such enclosed-type operations. Manure management, however, must still be considered in relation to ranch location and the decision must be made to use manures for fertilizer value

or to consider the manure as a true waste product.

For example, several non-enclosed poultry ranches have turned to liquid systems which utilize a wash-down manure management method that provides for recirculation of water from aerobic manure holding ponds in order to daily clean new poultry droppings atop concrete floors.

Also, enclosed, fan-ventilated deep-pit, poultry cage-housing can be successfully built and managed to the pleasure of both the farmer and surrounding suburbanites. Poultry manures have been retained inside these houses for as long as 2 years without the escape of FOD nuisances to the environment. The same is true for swine farrowing houses where space for rearing as many as 600 hogs has been accomplished on slotted floors atop liquid manure pits contained in above or underground structures. In either type of housing, however, the liquid system is designed for irrigation of adjacent cropland. It is interesting to note that in some areas, the most profitable practice for the average hog feeder is to dispose of the liquid manure in a lagoon and to use commercial fertilizer on his cropland (15).

Use of animal manures

Studies on retention of livestock and poultry manures for fertilizer value have always placed high in the list of priority projects. More commonly the results have demonstrated the value of animal manures for NPK soil requirements. Results from recent studies have shown the value of poultry manure for application to phosphorus deficient soils instead of trying to use this type of animal manure for nitrogen value alone (11). Also, recent land reclamation studies in California have shown that there is still another source for the use of dairy and poultry manures. Greater response was obtained from planted grasses and legumes in marginal lands treated with these fertilizers over a 5-year production period. In Connecticut, as well, a method of "maxi-mixing" which combines a large amount of poultry manure with a relatively small amount of soil placed in shallow earth basins provided in a period of 5 weeks a rich humus soil type which could be used in the growing of different field crops.

Research has not overlooked the value of recycling manures as an animal feed ingredient. Results from recent studies have shown overall values of poultry production when test flocks were fed rations containing as high as 40% dehydrated droppings. Likewise, commercial feeding trials in beef cattle have shown that poultry droppings could be used in livestock rations as a safe and cheap source of protein. In England, "Toplan" is the tradename for processed poultry manure fed to milk cows as a 25% mix and to beef or sheep as 50% mixes with barley in their daily rations.

Another development in recycling has been the use of dairy manure as a bedding material for cows maintained in open loafing barns. A rapid forced air composting system built within an open concrete-walled pit can be profitably operated on the same dairy where some, but not all of the manure can be repeatedly used for bedding purposes.

CONCLUSIONS

In conclusion, we have solutions for the control of FOD problems, we have methods by which we can manage, process, and use animal manures, but we lack the overall solution to the common denominator facing individual livestock and industry-wide operations—money. Thus, the outlook for the future is discouraging economically. Conditions can only get worse as stricter control is demanded of FOD problems and as anti-pollution ordinances are increased and tightened.

It appears that a price tag is needed on nuisance and pollution control methods. We need to define these costs as separate items in livestock operational expenditures instead of burying the figures under miscellaneous expenses. In this manner livestock associations can rightfully incorporate such costs into the price of their farm product much the same as other industries pass rising costs back to the consumer. Another possibility would be to increase depreciation values of farm equipment and structures in order to shorten the time to regain original investment costs.

This important segment of agricultural economics must be supported in order to backstop as well as to accelerate the progress made by the various livestock and poultry industries during the previous 20 years.

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NOTICE: All Secretary-Treasurers, please send in correct Officer list if above is incorrect.

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NOTICE TO MEMBERSHIP

In accordance with our Constitution and By-laws which requires our Second Vice-President and Secretary-Treasurer to be elected by mail ballot, you are hereby notified that President Walter M. Wilson, at the annual meeting in Milwaukee, Wisconsin, August 1972, appointed Roy Ginn, Chairman, Jack Gould, C. K. Johns, Irwin Gadd and Russel Helen-smith to the nominating committee for 1973. Nominations for the office of Second Vice-President and Secretary-Treasurer are now open and any member wishing to make a nomination should send a picture and biographical sketch of his nominee to Roy Ginn not later than March 1, 1973.

Roy Ginn, Chairman,
Nominating Committee
2353 N. Rice St.,
St. Paul, Minn. 55108

ANNOUNCEMENT CONCERNING THE SANITARIANS AWARD FOR 1973

Announcement is made that nominations will be accepted for the annual Sanitarians Award until June 1, 1973, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomin-

ation of individuals whose professional work in the field of milk, food, or environmental sanitation has been outstanding.

The Award consists of a Certificate of Citation and \$1,000 in cash, and is sponsored jointly by the Diversey Chemical Corporation, Klenzade Products, Inc., and Pennwalt Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 60th annual meeting of the Association which is to be held at Rochester, N. Y., in August 1973.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarians Award.

Eligibility:

1. *General Criteria*

To be eligible for nomination the Sanitarians Award offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

- a. Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;
- b. Be a living citizen of the United States or

Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation by a county, municipality, state or federal government provided that in the odd years beginning with 1969 the Sanitarians Award will be limited to state and federal employees and the even years to county and municipal employees.

Members of the Executive Board, members of the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, and industry members shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

- c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district, state or federal government with the United States or Canada.
- d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.

2. *Additional Criteria*

- a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.
- b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.
- c. No person who has received, or shared in receipt of the Award, shall be eligible for re-nomination for this Award.

Nominations

Nominations of candidates for the Sanitarians Award may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the sponsoring companies. Nominations from persons who are not members of the Association cannot be accepted. No member or Affiliate may nominate more than one candidate in any given year.

Each nomination must be accompanied by factual information concerning the candidate, a resume of his work and achievements, evidence supporting his achievements and if available, reprints of publications. A form for the submission of nominations may

be obtained upon request from H. L. Thomasson, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc., P. O. Box 437, Shelbyville, Indiana 46176.

Submission of Nominations

The deadline for submission of nominations is set annually, and all nominations and supporting evidence must be postmarked prior to midnight of that date. The deadline this year is June 1, 1973. Nominations should be submitted to Dick B. Whitehead, Chairman, Committee on Recognition and Awards.

Selection of the Recipient

The Committee on Recognition and Awards of the International Association of Milk, Food and Environmental Sanitarians, Inc., has full responsibility for selecting from among the candidates nominated the recipient of the Sanitarians Award. In judging the contributions of each candidate, the Committee will give special consideration to (a) originality of thought, mode of planning, and techniques employed, (b) the comprehensive nature of the candidate's achievements, and (c) their relative value as they affect the health and welfare of the area served by the candidate. The Committee will give consideration also to the efforts of the candidate to establish professional recognition in the area in which he serves, as well as to his research, administrative development, program operation and educational achievements. Additional information or verification of submitted information will be requested when considered necessary by the Committee. Testimonial letters in behalf of a candidate are not desired.

If after reviewing the nominations and supporting evidence, the Committee decides that the work and achievements of none of the candidates have been significantly outstanding, the Award shall not be made. In this connection, it is fundamental that if meritorious professional achievement cannot be discerned the Award shall be omitted for a year rather than to lower the standards for selections of a recipient.

Dick B. Whitehead
Div. of Occupational Health
Miss. State Board of Health
P. O. Box 1700
Jackson, Miss. 39205

VIRGINIA MEMBERSHIP COMMITTEES HOLD TWO SPECIAL MEETINGS

Mr. M. R. Cooper, Chairman of the Virginia IAMFES membership committee has been working with the executive and membership committees of the Virginia Association of Sanitarians and Dairy Fieldmen. They held a special meeting in conjunction

with the annual meeting of the Virginia Association at Blacksburg. At the membership committee meeting ways were discussed to involve more of their members in recruiting. The committee identified the following groups of workers as eligible to approach about membership, and set a goal of a 20% increase in membership in 1973. The committee intends to contact the following: (a) dairy industry personnel, (b) past members who have dropped out, (c) food industry personnel, (d) food regulatory personnel, (e) extension workers, (f) teachers active in agricultural and food fields, (g) laboratory personnel, (h) drug industry personnel, (i) drug regulatory personnel, (j) vo-ag teachers, and (k) dealers and service personnel.

The committee plans to meet again at the Virginia State Dairymen's Association convention at Fredricksburg in January. All members of the committee are looking forward to a substantial increase in new members during 1973.

TO ALL MEMBERS OF THE INTERNATIONAL ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS

The 1972 Federal Water Pollution Act has now been enacted into law. This is a tough new law backed by a very substantial national budget that not only mandates an accelerated pollution abatement program but also delegates broad enforcement powers to the Environmental Protection Agency.

We are sure that you are concerned how public health authorities, milk sanitarians and others who have traditionally been responsible for enforcement of our food purity laws will be effected by this new law.

We feel that your first step is to contact the E.P.A. officials in your area to offer your assistance and cooperation in their mandated responsibility under this new law.

Two parts of this new legislation are of prime interest to us:

1. The E.P.A. has authority to require point source compliance with certain water quality standards.
2. The E.P.A. now preempts the former right of states to set minimum standards on effluents discharged into the nations waters. States can, however, adopt more stringent regulations than the federal minimum.

As we understand it, the E.P.A. will, during the next few months, be gathering data to upgrade and validate previous technical and cost information. Later they will issue mandatory regulations.

In a matter of months E.P.A. approved permits will be required for those discharging polluted water. Permits issued by states may be subject to the E.P.A. veto if they do not meet federal affluent guidelines.

E.P.A. permits will specifically spell out what steps must be taken and set forth a timetable for completion of each step.

The first phase of the legislation mandates achieving the "best practical control technology" by July 1, 1977. The "best available technology", must be supplied by mid 1983.

Congress has authorized (but not yet appropriated) an expenditure of 24 billion dollars over the next three years, the majority of this will go for federal grants to municipalities for construction of sewage treatment facilities.

Such huge expenditures of money can not help but focus public attention toward purification of the nations waters.

The members of our association have the expertise, experience and organization to be of valuable assistance to the E.P.A. in fulfilling this new responsibility given them by Congress.

We, therefore, resolve as members of the International Milk, Food and Environmental Sanitarians Inc. that we offer our assistance and cooperation to the area E.P.A. officials.

In addition, that our president Mr. Walter Wilson write William D. Ruckelshaus, Administrator of E.P.A., Washington, D. C. 20460, offering consultation services of the International Milk, Food and Environmental Sanitarians through their Farm Methods Committee.

A. K. Saunders
Farm Methods Committee Chairman
R. E. Lock
Task Committee Chairman
Disposal of Animal Waste

SPEER NAMED TO COMMITTEE ON WEIGHTS AND MEASURES

John F. Speer, Jr., a dairy processor association executive, has been appointed to a five year term on an important committee of the National Conference on Weights and Measures.

Mr. Speer, executive assistant on the staff of the Milk Industry Foundation and the International Association of Ice Cream Manufacturers, will serve on the Conference's federal government liason committee. The Conference is an organization of weights and measures enforcement officials of state, county and city governments in the United States. It develops and adopts model laws and regulations, technical codes for weighing and measuring devices used in commerce, and tests methods and enforcement procedures.

Major function of the liason committee on which Mr. Speer will serve is to represent the Conference on all matters dealing with the Federal government and to make recommendations on matters concerning the relationships of Conference members, particularly with the national Bureau of Standards. The committee was instrumental in the promulgation of uniform state and federal regulations in connection with the Fair Packaging and Labeling Act. It works primarily with the Food and Drug Administration, the Federal Trade Commission, the U. S. Department of Agriculture and the U. S. Treasury.

DFI NAMES BRONSON LANE TO HEAD NUTRITION EDUCATION PROGRAM

Dr. C. Bronson Lane has been named Executive Secretary of the statewide Nutrition Education Council, a new division of Dairy Farmers, Inc.

Announcement of Lane's appointment was made by L. E. "Red" Larson, President of Dairy Farm-

ers, Inc., which represents the four major milk cooperatives in Florida.

Lane, a former Associate Professor of Dairy Science and Associate Extension Dairy Technologist at the University of Florida and University of Kentucky, will be based in Orlando in the DFI offices at 5600 Diplomat Circle. His staff is to include program directors qualified in the field of nutrition education who will operate out of Orlando, Jacksonville, Tampa and Miami.

"This program, statewide in scope and funded by the members of Dairy Farmers, Inc. is aimed at updating the nutrition education program in the state through the public and private school systems," Larson said. "In addition, Dr. Lane and his staff will work with the dental and medical associations in an effort to disseminate to the public at large accurate information on nutrition."

KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC. ANNUAL MEETING

The 1973 Educational Conference for Fieldmen and Sanitarians will be held, February 27-28, 1973, at the Executive Inn Motor Hotel, Louisville Kentucky.

All county and state health department personnel

(sanitarians, administrators and health officers) and milk and food industry fieldmen, plant managers and related service companies and university personnel are invited to attend.

The conference is sponsored by the Kentucky Association of Milk, Food and Environmental Sanitarians with assistance from the Kentucky State Health Department's Division of Environmental Service, the Kentucky Dairy Products Association and the Kentucky Dairy Association.

The program will be broken into general sessions, food and environmental sanitarians section, and milk sections.

Also, an awards luncheon will be held at the close of the meeting at which time awards will be presented to the Outstanding Sanitarian, Outstanding Fieldman and Outstanding Service Award.

Program topics to be included are as follows: Principles of Leadership, Reorganization of State Government, FHA — Water and Sewer Districts, Recreational Vehicle Park Sanitation, Development of Food Equipment Standards, Food Service Sanitation, Photography — An Inspectors Aid, Role of Communications Today, Sanitation Routing, Quality Forage for Quality Feeds, Milk Plant Waste Disposal, Cleaners and Cleaning Equipment.

Contact: Leon Townsend, Secretary-Treasurer, 110 Tecumseh Trail, Frankfort, Kentucky 40601.

REPORT OF THE EDITOR JOURNAL OF MILK AND FOOD TECHNOLOGY 1971-1972

REVIEW OF VOLUME THIRTY-FOUR

The December, 1971 issue completed publication of volume 34 of the *Journal of Milk and Food Technology*. Volume 34 established some new records for the *Journal* and equalled some of those established by volume 33. Volume 34 was the largest ever published. Its 728 pages exceeded those of volume 33 by approximately 6% and those of volume 30 published in 1967 by approximately 42%. The total number of papers (102) and the number of research papers (67) published in 1971 were about the same as in 1970. There were increases over 1970 in the number of technical general interest papers published and in the number of pages devoted to standards for equipment. In fact, 70 pages (or 9.6%) of volume 34 were devoted to information about equipment.

The portion of the *Journal* devoted to nontechnical material decreased somewhat in 1971. Eleven nontechnical general interest papers were published which is somewhat less than appeared in other recent volumes. Space devoted to Association Affairs and News and Events also declined in 1971, largely because of the pressure for publication of high quality scientific material. Details on the composition of volume 34 and a comparison with other recently published volumes can be found in Table 1.

The continued increase in the proportion of the *Journal* devoted to technical information of lasting value should be

welcomed by all readers, regardless of their positions and duties since all of them must make decisions based on current technical developments. An increase in up-to-date technical information better equips readers of the *Journal* to make the needed decisions.

Volume 34 again contained papers on a variety of subjects. Approximately 47% of the 102 papers were concerned with dairy foods and 46% dealt with other foods. The remaining 7% considered environmental or other topics.

PRESENT STATUS OF VOLUME THIRTY-FIVE

The first six issues of volume 35 have given readers as many pages of reading material as did the 12 issues of volume 30 published in 1967! In fact, four of the first six issues of volume 35 each contained 72 pages. These were the largest issues of the *Journal* ever published. The first six issues of volume 35 contained 41 research papers, 19 technical general interest papers, and 7 nontechnical general interest papers. This compares with 36, 11, and 4 in the same categories for the first six issues of volume 34. Beginning with the March, 1972 issue the *Journal* appeared with a new bright and bold cover which has been well received.

On July 15, 1972 there was a backlog of 55 papers awaiting publication. This included 28 research papers, 19 techni-

TABLE 1. SUMMARY OF CONTENTS OF THE *Journal of Milk and Food Technology*, 1967-1971

| Item | Volume 30 (1967) | Volume 31 (1968) | Volume 32 (1969) | Volume 33 (1970) | Volume 34 (1971) |
|---|---------------------|---------------------|---------------------|---------------------|---------------------|
| 1. Total pages, including covers | 512 | 540 | 624 | 688 | 728 |
| 2. Research papers | | | | | |
| a. Number | 30 | 32 | 47 | 66 | 67 |
| b. Pages | 137 | 142 | 205 | 280 | 288 |
| c. Percent of total pages | 26.7 | 26.5 | 32.9 | 40.7 | 39.5 |
| 3. General interest papers-technical | | | | | |
| a. Number | 11 | 16 | 14 | 18 | 24 |
| b. Pages | 47 | 74 | 87 | 99 | 150 |
| c. Percent of total pages | 9.2 | 13.7 | 12.2 | 14.3 | 20.6 |
| 4. Equipment standards | | | | | |
| a. 3-A, pages | 9 | 22 | 12 | 44 | 40 |
| b. E-3-A, pages | — | — | 7 ^a | 16 | 30 |
| c. Percent of total pages, all standards | 1.7 | 4.1 | 3.0 | 8.7 | 9.6 |
| 5. General interest papers-nontechnical | | | | | |
| a. Number | 23 | 14 | 26 | 20 | 11 |
| b. Pages | 72 | 65 | 91 | 64 | 46 |
| c. Percent of total pages | 14.1 | 12.0 | 14.6 | 9.3 | 6.3 |
| 6. Association affairs | | | | | |
| a. Pages | 64 | 68 | 62 | 49 | 45 |
| b. Percent of total pages | 12.5 | 12.6 | 9.9 | 7.2 | 6.3 |
| 7. News and events | | | | | |
| a. Pages | 51 | 42 | 36 | 23 | 17 |
| b. Percent of total pages | 9.9 | 7.8 | 5.8 | 3.4 | 2.3 |
| Percent of pages-Technical material incl. standards | 37.6 | 44.1 | 48.1 | 63.7 | 69.7 |
| Percent of pages-Nontechnical material | 36.5 | 32.4 | 30.3 | 19.9 | 14.9 |
| Percent of pages-Covers, ads, index, etc. | 25.9 | 23.5 | 21.6 | 16.4 | 15.4 |

^aThese were Baking Industry Equipment Standards

cal general interest papers, and 8 nontechnical general interest papers. In addition, on July 15 there were 14 research papers, 2 technical general interest papers, and 1 nontechnical general interest paper being reviewed or revised. Although the backlog of papers awaiting publication is somewhat higher than at the same time in 1971, it is down considerably from the high of approximately 90 papers early in 1972. We are still able to publish research papers in six months or less after they have been received.

REVIEW PAPERS

Review papers on timely topics continued to appear in volume 34 and the first one-half of volume 35. Subjects covered by these reviews include: microbiology of poultry products, changing patterns in food production and processing, solid waste disposal, bacteriological testing of milk, fecal contamination of fruits and vegetables, progress in the cheese industry, production of noncarcinogenic food additives, microwave ovens, *Vibrio parahaemolyticus* food poisoning, plastic packages and the environment, rodents in the environment, staphylococcus food poisoning, perfringens enterotoxin, sodium in foods, lactose, isolation of *Clostridium perfringens*, lead poisoning, ecology of milk packaging, trends in dairy foods, ecology of the lactic streptococci, *Bacillus cereus* food poisoning, automation in the dairy laboratory, the propionic acid bacteria, interactions between starter cultures and food-borne pathogens, importance of diet in heart disease, and quality control in the California wine industry.

Subjects of review papers awaiting publication include: microbiology of meats, emerging food-borne diseases, botulism and staphylococci in meat products, public health aspects of barbecued foods, relationship between lactic acid bacteria

and human and animal health, and cooling of foods.

In addition, review papers on the following topics have been promised: process cheese, nitrates and nitrites in meats, mercury in foods and the environment, water activity, *Vibrio parahaemolyticus*, milk flavor, abnormal milk and milk processing, Swiss cheese flavor, and viruses in foods. The Editor continues to believe that review papers are valuable contributions so that busy people can be aware of developments in fields which are related to but are not their immediate concerns. Well written reviews on timely topics are always welcomed and will be published promptly.

EDITORIAL BOARD

During 1971 two long-time members of the Editorial Board resigned because they retired from professional activities. They are C. A. Abele and Dr. W. S. Mueller. Three scientists were added to the Editorial Board so it now consists of 42 scientists in governmental, industrial, and university laboratories in the U.S. and Canada. Members of the Editorial Board appointed in 1971 include: Drs. S. E. Gilliland, R. V. Lechowich, and H. Pivnick. In addition to the Editorial Board, the following served as special reviewers during the first six months of 1972: Drs. D. B. Lund, R. L. Bradley, Jr., T. E. Minor, J. H. von Elbe, W. J. Hausler, Jr., and A. J. Maurer. Their help is acknowledged and appreciated.

IMPACT OF THE JOURNAL

An Editor is concerned with: (a) the content and quality of each issue of the *Journal*, (b) whether the *Journal* is being read, and (c) whether the *Journal* makes an impact on the reader. The first two of these concerns are easiest to measure

but the last is more difficult to determine although it is very important.

One measure of impact might be how widespread abstracts of papers in the *Journal* appear in secondary information sources. Abstracts of appropriate *Journal* papers regularly appear in: *Chemical Abstracts*, *Biological Abstracts*, *Food Science and Technology Abstracts*, *Microbiological Abstracts*, *Dairy Science Abstracts*, the abstracts section of *Milchwissenschaft*, *Commercial Fisheries Abstracts*, *Cheese Abstracts*, *Dairy and Ice Cream Field*, and a Russian abstract journal. Furthermore, the table of contents of each issue appears in *Current Contents* within a month or two of publication. Undoubtedly abstracts appear in still other publications but this list is sufficient to indicate the wide dissemination of information published in the *Journal* and hence the great opportunity for impact.

Another measure of impact is the frequency with which *Journal* papers are cited as references in books, in other papers in the *Journal*, or in papers which appear in other journals. This, of course, is difficult to determine because of the time involved in checking through other journals. However, the 13th edition of *Standard Methods for the Examination of Dairy Products* was just published. There are 473 references in the book and 107 or 23% of these are papers which appeared in the *Journal of Milk and Food Technology*. The *Journal* truly has had a remarkable impact on this book and hence on the professional lives of persons who use the book.

The final, and perhaps the best criterion of impact is the reader himself. Has he gained new insights from the *Journal*? Has he been motivated to take some action? Impact here will vary with the individual reader, his interests, and the time he devotes to professional growth and development and is almost impossible to measure. Although, without doubt, the *Journal* has had considerable impact in some areas in the past, continued efforts are needed to expand this impact and hence that of IAMFES.

Respectfully submitted,
 E. H. MARTH
 Editor,
Journal of Milk and Food Technology

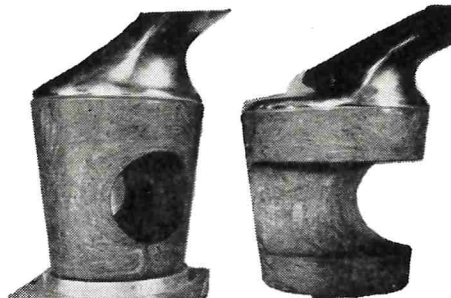
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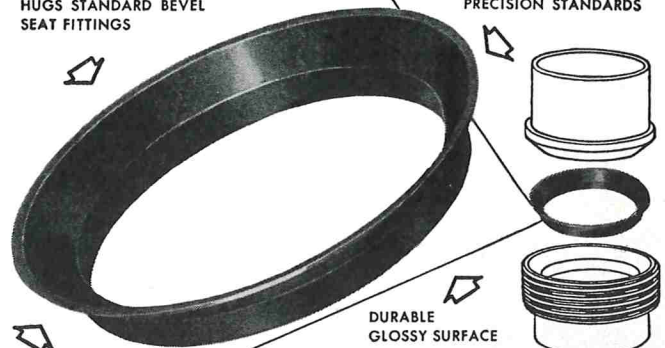
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Dairy authorities speak out
on better cow milking.



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Prime the udder for maximum milk let-down

Proper pre-milking stimulation is the first and a most important step in the milking operation. It increases milking rate, cuts labor cost and brings about maximum milk ejection which helps maintain udder health.

Production of high quality milk demands that the udders be cleaned and sanitized. Fortunately, the cleaning process can accomplish both sanitation and stimulation.

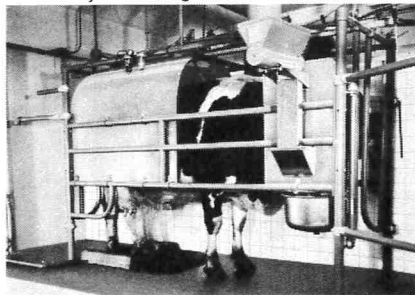
Washing or massaging the udder causes a stimulation of the nerve endings, releasing a hormone called oxytocin from the pituitary gland. In the udder, oxytocin causes a contraction of the muscle tissue thus expelling milk into larger ducts and cisterns. A recent experiment demonstrated that the sounds of milking do not cause adequate release of oxytocin to achieve milk let-down.

It has been determined that the response to oxytocin will begin 13 to 50 seconds after washing and massaging the udder and will last for 2 to 10 minutes, depending on the quantity released. The act of stimulating should be performed one or two minutes before the milking machine is attached. This interval is of the utmost importance. If a cow is stimulated 5 to 10 minutes before the milking machine is attached, most of the effect of oxytocin will be lost. Results of work at Kansas have shown that when milking was delayed 8 minutes after stimulation, yields decreased by 5% and machine time increased by 14%.

A milking machine should not be placed on a cow until her teats are

plump and fully distended from the pressure of the milk. Teat cups will draw in flabby teats and loose udder tissue when milk pressure is low. If this happens, the teat can be injured or partially blocked and milking time will be longer.

A cow in the early lactational phase will often only require a 10 second stimulation period to achieve full let-down, whereas cows in the latter half of lactation will often require 50 seconds or more. When all cows are compared, the higher producers generally require less stimulation. However, even with high-producing cows, merely brushing the udder to remove



dirt or simply using a strip cup does not prove adequate to stimulate maximum let-down. In a New Zealand study, one identical twin in each of several pairs was stimulated for 30 seconds before milking. The unstimulated twin cows exhibited a rapid decline in milk production after 50 days, allowing the stimulated twins to produce 32% more milk and fat.

From a milking rate study, conducted by Dr. J. D. Sikes and the author at the University of Missouri, it was learned that total parlor time decreased by approximately 2 hours per day when 79 cows were properly stimulated by an automatic stimulator-washer. The results of this experiment are shown in the table.

PERCENTAGES OF COWS THAT MILKED OUT IN VARIOUS INTERVALS

| | Manual (15 Sec.) | Automatic (30 Sec.) |
|----------|---------------------|------------------------|
| 0-3 min. | 0 | 15.3 |
| 3-4 min. | 9.8 | 45.8 |
| 4-5 min. | 33.3 | 26.4 |
| 5-6 min. | 16.7 | 9.4 |
| +6 min. | 40.3 | 2.8 |

Milking time per cow decreased with the use of the stimulator-washer. Cows prepared with the stimulator-washer ranged from 2.02 to 7.47 minutes, while cows prepared manually required 3.40 to 14.7 minutes for milking. On the average, cows that were stimulated for 30 seconds gave 76% of their milk in the first two minutes as compared with only 51% with a 15 second pre-milking stimulation. Milk yield in the first 30-60 seconds of milking serves as a measure of completeness of milk ejection.

In this experiment, an automatic stimulator-washer was used. It should be noted, however, that careful and thorough hand-stimulation can achieve the same results.

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