STUDIES ON STAPHYLOCOCCUS ENTEROTOXIN

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37.

Studies on Staphylococcus Enterotoxin.

Introduction.

Within recent years, voluminous reports have appeared dealing with the divers toxic properties of filtrates, prepared under suitable conditions, from certain strains of staphylococcus. The complexity of these filtrates was further illustrated by the demonstration of a gastro-intestinal irritant in filtered broth preparations from strains implicated in outbreaks of food-poisoning. With the recognition of this micro-organism as a potential etiological agent of gastro-enteritis, a number of food-poisoning epidemics were reported in which staphylococcus was incriminated.

As early as 1907, Owen (1) described an outbreak in which 19 persons suffered from acute gastro-enteritis following a meal of dried beef, contaminated by large numbers of staphylococci. A milk-borne epidemic was reported by Barber (2) in the Philippines in 1914. He observed acute gastro-intestinal distress in a number of persons who had ingested milk contaminated by an albus strain of staphylococcus. In this case, more conclusive evidence was produced before the causative micro-organism was incriminated. Barber inoculated the organism into sterile milk, incubated it at 36.5° C. for 8½ hours, and drank a 50.0 c.c. portion. Acute gastro-enteritis, similar in nature to that observed in the other persons afflicted, followed within 2 hours. A portion of the milk, refrigerated immediately

following inoculation for the same length of time, did not cause symptoms of food-poisoning when ingested. The organism was isolated in almost pure culture from the udder of the cow producing the milk. Barber's classic report presented the first conclusive evidence that food-poisoning could result from staphylococcal contamination.

This work remained unconfirmed for some 16 years, until Dack and his colleagues (3) investigated a similar outbreak due to the elaboration of a filtrable poison by a yellow hemolytic staphylococcus, in a cream-filled Christmas cake. Portions of the cake were ingested by volunteers, and acute gastro-enteritis followed within 3 hours. A 40-hour broth filtrate of the yellow staphylococcus isolated from the cake produced identical symptoms when ingested by volunteers. The poisonous substance appeared to be filtrable entexotoxin, causing a food intoxication.

Following the latter publication, there appeared in rapid succession numerous observations of food-poisoning attributed to the growth of staphylococcus in food products. (Table 1.) A study of the literature shows a uniform sequence of symptoms, characterized by a short but definite incubation period of 2 to 4 hours following ingestion of the contaminated food. Towards the end of the incubation or latent period, nausea and dizziness appear, accompanied by sweating, chilliness and salivation. The nausea increases in intensity and rapidly gives way to

violent paroxysmal vomiting, abdominal cramps, diarrhoea and
prostration; there may be tetanic muscular contractions and
intense muscular pain. The more acute symptoms usually pass
away within a few hours, but general weakness may persist for
several days. It is not unusual however, for the patient to
feel normal within 24 hours after onset of the illness. These
symptoms have been faithfully reproduced on a number of occas-
ions when sterile broth filtrates of suspected strains of
staphylococcus have been ingested by human volunteers. (3, 10,
14, 22.)

A review of available data reveals that there have been
recorded at least 47 epidemics attributable to the presence of
this micro-organism in food stuffs. Of these 47 reported out-
breaks, 40 have involved some 1400 persons. (Table 2.) It is
a matter of some significance that this type of food-poisoning
should show a predilection for milk and milk products. The
report of Barber (2) in 1914 has already been recounted.
Ramsey and Tracy in 1931 (4) described an epidemic attributed
to the presence of an orange staphylococcus. They noted an
"off flavor" described as a "malt" or"caramel" flavor in the
milk in which the organism was grown. Tanner and Ramsey, (5)
working with the same organism at a later date, stated that
several of a group of 20 individuals visiting the laboratory
to test the "off flavor" were made ill. It is of interest to
note in this connection, that a stronglyhemolytic golden
staphylococcus, obtained from the Department of Dairying at the

~~at the~~ University of British Columbia, and reputed to produce
a "caramel flavor" when grown in milk, elaborates a powerful
gastro-intestinal irritant.

A list of the foods implicated in the reported outbreaks
shows that of 47 epidemics recorded, 27, or 57% were traced to
milk and milk products. (Table 2.) Reference to certain pub-
lications might tend to explain, in part, the frequent impli-
cation of this type of food. Stark (6), in 1926, examined
bacteriologically 25 certified dairy cows. The milk from every
animal revealed white staphylococci, nine strains of which were
hemolytic. In a series of 260 cattle examined by Gwatkin and
his colleagues, (7) 143 had mastitis, and 30 of these were due
to staphylococcus. 67.4% of the strains isolated from these
cows were toxin producers.[†] 20% of strains <u>from normal udders</u>
also produced toxin. Crabtree and Litterer (8) reported a
milk-borne epidemic due to a hemolytic staphylococcus from
mastitis in two cows. Over 242 cases occurred amongst members
of a school before the cows were singled out from the school
herd. Of 8 strains isolated at random from raw milk bottled
for local distribution, and tested in this laboratory, 6 pro-
duced a gastro-intestinal irritant.

While cream pies, chocolate éclairs and custard-filled
pastries appear to provide a singularly favorable medium for
growth and consequent toxin production by this organism, and

[†] Toxin in Gwatkin's report refers to hemolysins.

have been held responsible for a large number of outbreaks,
(Table 2.) it is inconceivable that all of these could be
traced to a contaminated milk supply. Kellert (9) reported an
epidemic caused by bakery goods, contaminated from a boil on a
baker's forearm. Investigations on strains isolated from milk,
food epidemiologically implicated, pathological lesions and a
number of other sources led Jordan (10) to believe that a high
percentage of these organisms are capable of elaborating a
gastro-intestinal irritant. A strain "B", used in this lab-
oratory for a greater part of the experimental work caused an
erythema in the nose of the person from which it was isolated.
It has produced a powerful enterotoxin of undiminished potency
over a period of a year. The fact that strains from sources
other than contaminated milk supplies may cause food-poisoning
is further substantiated by reports of epidemics due to growth
of staphylococcus in chicken gravy (11), ham (12), sweet
potato candy (12), and tongue sandwiches (13).

With the recognition of increasing numbers of this type
of poisoning arose the necessity for a laboratory test by means
of which those strains producing an enterotoxin could be read-
ily recognized. In many of the earlier epidemics, the strains
concerned were tested on human volunteers, or their incrimi-
nation remained unconfirmed, and they were merely suspected.
In his investigations in 1930, Jordan (10) used human volunteers
and it was clearly evident that for a number of reasons the
human was neither a convenient nor satisfactory laboratory

animal. A recent paper by Dack et al. (14) reports feeding
experiments on a number of humans. It would indicate that
individual susceptibility varies considerably, and inasmuch as
extreme care must be exercised in administering the toxin, the
dosages are necessarily so small as to not show typical re-
actions in many instances. Furthermore, the number of vol-
unteers available is frequently limited to such an extent that
experiments performed are scarcely of significance.

The use of M. rhesus monkeys, first reported by Jordan and
McBroom in 1931 (15), has since been confirmed by Woolpert and
Dack (16). Similar difficulties arise here as in the case of
the human volunteer. Meyer (17), in 1934, stated; "Contrary
to experiences made by Jordan and McBroom, and Woolpert and
Dack, feeding tests on monkeys have yielded inconclusive
results." Furthermore, monkeys are difficult to handle, and
an experiment on even a moderate scale would be expensively
impractical.

Dack and his colleagues (3) inoculated filtrates intra-
venously into rabbits. Complications that arose due to the
presence of the hemolytic, lethal and other toxic substances
caused the death of several animals; vomiting was not observed,
although loose stools in some cases were recorded. There was
obviously no specificity in such a test and it was abandoned.
Borthwick, in 1933 (18), reported a method of preparation that
rendered guinea pigs and rabbits suitable for ingestion exper-

iments on the enterotoxin. No vomiting or diarrhoea occurred, and the post-mortem findings strongly suggested death from the other exotoxic products of staphylococcus. Attempts made in this laboratory to reproduce the results have so far been un-successful.

A detailed study of the agglutinative properties, biochem-ical reactions and chromogenicity of a large number of strains implicated in food-poisoning outbreaks (27) failed to reveal any constant characteristics by which those organisms produc-ing enterotoxin could be recognized.

A cultural method for detecting food-poisoning strains of staphylococci has been described by Stone (19). The suspected organisms are grown in a 3% beef-extract-gelatin medium. Liquefaction of the gelatin in 24 hours is taken as an indica-tion that a strain produces enterotoxin. A recent modifica-tion by the same author includes agar in the medium, and ammo-nium sulphate solution as a developer. The presence of clear zones around a colony indicates an enterotoxin producer. No confirmation of these results has been published, and the few experiments performed in this laboratory have yielded results inconcordant with animal and human tests.

Due to lack of suitable experimental animals and adequate tests, few conclusive statments have been made regarding the properties of this gastro-intestinal irritant. Jordan and Dack (20), and Jordan and Burrows (21), recently reported the

following characteristics:

1. The active principle will not distil .

2. It is not readily dialyzable.

3. It is markedly unstable to N/100 NaoH.

4. It is unstable to heat in N/100 H Cl solution.

5. It is not identical with the hemolytic substances present in many staphylococcus filtrates, nor does it produce a skin reaction.

6. It is completely removed from acid aqueous solution by extraction with ethyl ether or chloroform, as judged by our method of assay.

7. It may be extracted from alkaline solution with ethyl ether or chloroform but the deleterious effect of alkali tends to mask such removal.

8. The gastro-intestinal poison is not completely destroyed when exposed to a temperature of 100° C. for 30 minutes.

9. The toxic quality does not disappear after storage at low temperatures for as long as 67 days but is perhaps somewhat weakened.

Monkeys injected intravenously with 5.0 c.c. of saline solution of acid ether extract of potent filtrates vomited and manifested the usual signs of acute distress of the gastro-intestinal tract. The same solution given intravenously to guinea pigs, rabbits, cats and dogs produced no ill effects whatsoever.

Several workers agreed that no tolerance was induced by repeated ingestion of the food-poisoning substance. Woolpert and Dack in 1931 (16), stated that a degree of active immunity could be induced by repeated parenteral inoculation, but all attempts at passive immunity failed.

Woolpert and Dack (16), using monkeys as test animals, and Dolman (22), 1934, in human feeding experiments, demonstrated that the hemolysins and diverse other toxic properties of staphylococcal filtrates are distinct from the food-poisoning substance. In the latter's experiments, humans ingested relatively large amounts of filtrates containing potent hemolysins and their allied toxic substances with impunity, while much smaller amounts of filtrates prepared from one particular strain evoked symptoms of acute food-poisoning in several volunteers. This strain produces potent enterotoxin.

The lack of a convenient and specific test for the presence of the enterotoxin has hampered investigation of its chemical nature and antigenic structure. Consequently the conditions favoring its production, and its mode of action on the animal system are as yet undefined. Meyer (17), in a discussion on staphylococcus food-poisoning states; "However, in view of the well known fact that the enterotoxic substance is distinct from the killing toxin of a staphylococcus, it is not admissible to consider a given strain as an offender in a food-poisonig out-break without a direct demonstration of the gastro-intestinal

irritant. While heating greatly reduces the killing toxin, and leaves the enterotoxic capacity unimpaired, the separation of the two fractions is frequently fraught with difficulties. The tests on human volunteers are conclusive but not without risks. Suitable monkeys are not always available, and the preparations of small laboratory animals by the methods described by Borthwick are not always effective. A simple physiologic-pharmacologic test to detect and to separate the enterotoxic substance from the killing poison of a staphylococcus is, therefore, urgently needed."

The work undertaken in this laboratory was primarily an attempt to find a reliable biological test sufficiently convenient that it might be carried out in any bacteriological laboratory as a means of identification of those strains producing enterotoxin. It was further deemed desirable that the test be adequately sensitive in detecting the presence of the gastro-intestinal irritant that it might be employed in studying the various properties, mode of action and requirements for the production of the irritant substance.

Experimental.

The strain used in the greater part of our experimental work, designated as strain "B", was isolated from a human nasal mucous membrane, and caused a chronic erythema of that organ. Toxic filtrates of strain "B" contain potent α and β hemolysins, and their allied exotoxic products, and by virtue of this property were at one time included with the pooled preparations from a number of organisms during the process of producing staphylococcus toxoid at the Connaught Laboratories, Toronto.[†] Small subcutaneous doses of these toxoids caused nausea and vomiting in a number of human subjects; but when strain "B" was removed from the preparations the violence of the reactions diminshed. Ingestion experiments on human volunteers confirmed the suspected presence of enterotoxin, gastro-enteritis appearing within 2-2½ hours after consumption of as little as 1.0 c.c. A subcutaneous dose of 0.1 c.c. caused acute symptoms in a human within 1½ hours after inoculation; the patient did not completely recover for several days.

When strain "B" was reisolated one year later from the same patient's nose, it was shown to have retained its ability to produce a powerful gastro-intestinal poison. In this laboratory it has consistently produced an enterotoxin of undiminished potency for over one year. The stock-culture is maintained in beef-infusion agar.

† Personal communication from Dr. C.E. Dolman.

Preparation.

The method of preparation employed by Dolman (22), and Dolman and Kitching (24) in producing staphylococcus toxoid for clinical use is followed. Plates of semi-solid beef-infusion agar (0.3% agar) are inoculated with a five-hour broth culture of the organism. After 40 hours incubation at 37° C. in an atmosphere of 70% oxygen and 30% carbon dioxide, the contents of the plates are squeezed through cheese cloth into filtering funnels containing coarse filter paper. The filtrate is cent-rifugated at high speed for some $2\frac{1}{2}$-3 hours and sterilized by passing through a Seitz E.K. disk. The preparation from strain "B" contains potent hemolysins against rabbit and sheep ery-throcytes, has high lethal and dermo-necrotic powers as well as the various other toxic properties characteristic of staphy-lococcus filtrates. The α lysin (rabbit cell) may measure 2500 units and the β lysin (sheep cell) 1600 units as calibrated by the method of hemolysin titration described by Dolman (25). The removal of these toxic substances is effected by the addi-tion of 0.3% solution of formaldehyde U.S.P. and incubation at 37° C. until the α and β hemolysins are no longer detectable (24). One now has a filtrate devoid of the deleterious lethal hemo-lytic and dermo-necrotic properties, quantities up to 1.0 c.c. intraperitoneally occasioning no observable harm to mice. The preparation was tested for enterotoxin on a human volunteer, who ingested portions on two occasions.

Protocol 1.

L. 1.0 c.c. was ingested in 30 c.c. of milk. Four hours

later, nausea and headache, accompanied by dizziness appeared.
They passed away within one hour. Two hours later a very loose
and foul-smelling stool was passed. Recovery was complete
within a few hours.

Protocol 2.

2. 1.5 c.c. of the same filtrate was ingested in 30.0 c.c.
of milk. Two hours after ingestion of the preparation nausea
appeared, increased in intensity, and was accompanied by con-
siderable gastric discomfort and sweating. Within 30 minutes
of the appearance of nausea, violent paroxysmal vomiting
occurred, followed by extreme weakness and disability. Abdom-
inal pains and a general feeling of discomfort persisted for
some 2 hours following the vomiting, during which time 3 stools
were passed. They were almost entirely fluid, foul-smelling
and profuse. Abdominal discomfort was relieved with passage of
the stools but a feeling of nausea persisted for several hours.
The following day aversion to food, and general weakness were
evident. Recovery was complete within 48 hours.

These two experiments, combined with the reports of sim-

ilar instances cited above in relation to experiences with

strain "B" in the preparation of toxoids at the Connaught Lab-

oratories in Toronto indicated that (a) the enterotoxin was

more stable to 0.3% formalin at 37° C. than were the hemolytic,

dermo-necrotic and lethal properties. (b) The enterotoxin

was present in appreciable quantities in filtrates from which

the aforementioned properties had been entirely removed. This

method of separation of the gastro-intestinal irritant from the

killing, hemolytic and dermo-necrotic properties has been used almost

entirely in the case of strain "B" and some others with singular

success throughout the investigation.

The first point of investigation was to determine the effect of the filtrate <u>containing only the enterotoxic principle</u> on guinea pigs, using Borthwick's method of preparation (18). This procedure is outlined in brief.

The insusceptibility of rabbits and guinea pigs was attributed by Borthwick to the destruction of the gastro-intestinal irritant by the acid reaction of the stomach. Accordingly the stomach contents were adjusted to pH. 7.3 with NaHCO3 5.0% and N/10 HCl, titrations being carried out by means of a stomach tube. When the desired reaction was obtained, the toxic filtrate was introduced and the stomach tube withdrawn. Borthwick reported death in a number of animals within one hour of administration of the toxic substances.

In this laboratory, it was deemed advisable to use a concentrated phosphate buffer to obtain the desired pH. rather than carry out the somewhat lengthy procedure of titration.

An example of the method followed in standardizing the buffer is outlined.

Protocol 3.

Pig 1. 10 c.c. of K2HPO4, in a concentration of 80g. per litre, p.H. 8.83 were introduced into the stomach by means of a stomach tube. Small samples were aspirated at intervals of 5, 10,15, 20, minutes and the pH. of each tested on a quinhydrone electrode.

original p.H of buffer solution	8.83
Sample aspirated at 5 minutes	7.43
" " " 10 "	7.35
" " " " 15 "	7.30
" " " " 20 "	7.32

Protocol 4.

Pig 2. 15 c.c. of K2HPO4 in a concentration of 80g. per litre. p.H. 8.83, were introduced into the stomach. Samples were aspirated at 2, 5, 10, 15, minutes and the p.H. tested on a quinhydrone electrode.

original p.H. of buffer solution	8.83
Sample aspirated at 2 minutes	7.55
" " " 5 "	7.35
" " " 10 "	7.35
" " " 15 "	7.35

A suspension of MgCO3, 10 g. per litre, was also used as a buffer solution.

Protocol 5.

Pig 1. 12.5 c.c. MgCO3, (10g. per litre) were intro-
duced by means of a stomach tube. Samples were aspirated at
2, 5, 10, 15, 20 minutes.

Sample aspirated at	2 minutes	7.2
" "	" 5 "	7.43
" "	" 10 "	7.30
" "	" 15 "	7.30
" "	" 20 "	7.30

The toxic filtrate was administered about 2 minutes af-
ter the buffer, in quantities of 2.0 to 7.0 c.c. without prod-
ucing any symptoms other than slight nervous twitching.

A portion of filtrate "B" was concentrated to about
1/10 its original volume by distillation in vacuo at 37° C.
Doses equivalent to 70 and 80 c.c. were administered enter-
ally to guinea pigs without any untoward effect.

An abdominal incision was made in a guinea pig, and
2.0 c.c. of filtrate "B" introduced directly into the duo-
denum by means of a syringe. The incision was stitched. The
animal recovered from the anaesthetic within 20 minutes,
became active and remained so for a week, when it was des-
troyed. At no time did it exhibit symptoms attributable to
the presence of the toxic filtrate.

These experiments led one to believe that the p.H.
of the stomach might be of little importance; and indeed in
humans, it seems to provide no protection. One was interested
as to whether, if the toxin were absorbed from the gastro-
intestinal tract of the guinea-pigs, the characteristic mani-
festations of vomiting and diarrhoea were possible.

A guinea-pig was given intramuscularly, o.05 grains
of apomorphine. Slight salivation, but no vomiting occurred.
There was little evidence that the animal was in any way act-

-ting abnormally under this stimulus. Similarly, 0.05 mgm.
of histamine occasioned no upset in a guinea-pig. Large
quantities of phosphates administered as buffer solutions,
and also castor-oil produced/changes in the nature of the
insignifigant
stools.

One was prompted to believe that, if vomiting were
possible in a guinea-pig it occurred very rarely, and not
under the usual stimuli affecting humans. Thus, one of the
most characteristic symptoms of staphylococcus food-poisoning
would possibly be absent if this animal were used in lab-
oratory tests; and in the experience of the author, on no
occasion has a guinea-pig given any indication of showing
the usually prominent symptoms of gastro-enteritis. This
method therefore was abandoned.

Inasmuch as cats and dogs resemble humans more closely
than do rodents in their dietary and excretary habits, and
both are known to vomit on occasion, it seemed logical to
investigate the susceptibility of these animals to the gastro-
intestinal irritant.

Preliminary investigations were carried out on cats,
using the filtrate of strain "B" tested on a human. (Protocols
1 and 2.)

Protocol 6.

Cat No.1   2 months old, weight about one kilogram.
50.0 c.c. of filtrate "B" were administered in the animal's
milk when it was fed.
Time   12.35 P.M.
1 Hr.35 Min.   The animal became somnolent and drowsy; it
preferred to lie down and moved very little.

1 Hr.45Min. The cat became agitated. It licked its lips violently; salivation was very marked.
1 Hr.50 Min. The cat vomited. It seemed much relieved, became more active and played a little.
2 Hr. 05 Min. Vomiting was repeated; merely gastric secretions.
2 Hr. 15 Min. The cat became unsteady on its feet and refused food.
3 Hr. 10 Min. Giddiness was apparent; the animal swayed from side to side when it sat up.
3 Hr. 15 Min. Violent vomiting recurred, 3 times. The animal's condition seemed much improved after vomiting.
4 Hrs. The cat accepted food.
4 Hr. 15 Min. The animal was playing and seemed quite recovered. There was no signifigant diarrhoea; recovery was complete within 24 hours.

Protocol 7.

Cat No.2. 2 months old, weight about one kilogram.
50.0 c.c. of entero toxic filtrate were ingested in milk.
Time: 12:35 P.M.
1 Hr.55 Min. The animal regurgitated; there was some gastric distress. Salivation was evidenced by licking of the lips.
2 Hr.05 Min. Giddiness was apparent. The animal swayed dizzily when standing or sitting up.
2 Hr.40 Min. Gastric distress increased; violent peristaltic movements were observed through the abdominal wall.
3 Hr.05 Min. Another wave of nausea occurred, the animal shuddered spasmodically; violent peristaltic movements were observed through the abdominal wall. Nausea appeared to come at intervals.
3 Hr.25 Min. The general condition of the animal seemed improved.
4 Hr.30 Min. A large bulk of fluid feces, foul-smelling and light in color was passed. It was mucoid in consistency. The animal recovered completely within 24 hours.

Protocol 8.

Cat No. 3 Large female, fully grown.
6.0 to 8.0 c.c. of filtrate "B", concentrated by distillation in vacuo to about 1/10 volume, were given in milk. The dose was equivalent to 60-80 c.c. of the original filtrate.
Time 3.00 P.M.
2 Hrs. The animal remained quiet all afternoon. At 5.00 P.M. it became agitated, cried frequently and licked its lips.
2 Hrs.40 Min. It vomited violently. The animal seemed relieved after vomiting.
3 Hrs. Violent vomiting was repeated, and diarrhoea followed shortly after. A large bulk of foul-smelling feces, mucoid in consistency, was expelled. The animal recovered completely.

The above protocols, merely representative of a number of others in which the manifestations of acute gastro-intestinal distress were evident in greater or lesser intensity, are a complete picture of the symptoms of staphylococcus food-poisoning in humans. Vomiting occurred from 2 to $2\frac{1}{2}$ hours after ingestion of the filtrates, preceded by salivation and followed by abdominal distress and diarrhoea. Recovery was rapid and complete in all cases. As some workers have observed in humans, diarrhoea may be present without vomiting or vomiting may occur alone, although as a general rule both symptoms are marked.

The dose required to produce these typical manifestations of food-poisoning in cats when given enterally is about 50 times that required to cause vomiting and diarrhoea in humans on ingestion of the same filtrate. (Protocols No. 1 and 2) The enteral administration of such large doses to laboratory animals is neither convenient nor accurate. Inasmuch as the enterotoxic preparation used was innocuous, the hemolysins, lethal and dermo-necrotic substances having been previously destroyed by formalin, parenteral innoculation was employed.

Protocol 9.

Cat No. 1.   2-3 months old, weight 800-1000 grams.
3.0 c.c. of filtrate "B" were given intraperitoneally.
Time: 11:20 A.M.
15 Mins.   The animal vomited violently.
20 Mins.   Vomiting occurred several times.
30 Mins.   Repeated vomiting occurred.
40 Mins.   A very loose foul-smelling stool, mucoid in
            consistency was passed.
2 Hr. 55 Min. The animal appeared to be weak and somnolent.
3 Hr. 55 Min. The cat seemed dazed and giddy when standing or
            sitting up. It preferred to lie down, and was com-

-pletely disinterested in its surroundings.
4 Hr.20 Min. Signs of recovery were evident. The animal became more active and interested in it's surroundings. Complete recovery followed within 24 hours.

Protocol 10.

Cat No.2.   2-3 months old, weight about 1 kilogram.
1.5 c.c.filtrate "B" were given intraperitoneally.
Time: 2.30 P.M.
25 Mins.   The animal seemed nauseated, uncomfortable.
37 Mins.   The animal lay on the floor of the cage; its head hung unnaturally with the nose resting on the floor.
1 Hr.   Salivation, accompanied by licking of the lips was followed by repeated vomiting.
1 Hr.10 Mins.   Vomiting was repeated.
1 Hr. 25 Mins.   A loose foul-smelling stool was passed. It was profuse, mucoid in consistency and light colored. Retching and vomiting were repeated over a period of an hour, accompanied by several loose stools. The animal appeared weak and somnolent.
3 Hrs.30 Mins.   The cat seemed to be recovering, for it became more active and accepted food.
Recovery was complete in 24 hours.

Protocol 11.

Cat No.3   - 6 weeks old, weight 400 grams.
0.5 c.c. filtrate "B" were inoculated intraperitoneally.
Time: 2:07
15 Mins. Vomiting occurred.
23 Mins.   Vomiting was repeated.
24 Mins.   Vomiting was repeated.   The animal remained somnolent and lethargic up to 6.00 P.M. Diarrhoea occurred some time later.

It will be observed that in parenteral inoculation of

filtrate "B", the syndrome is merely an intensified form of

that occurring when enteral administration is employed. The

latent period is shortened from 2-3 hours to as little as 5-10

minutes, depending in part, upon the size of the dose ad-

ministered.   The symptoms, in the author's experience, are

more violent and definite.   An increased rate of respiration

and even panting has been observed, prior to the onset of

vomiting.   Salivation is marked; the vomiting is projectile

and recurs at frequent intervals. Stools are frequent, and after the first one or two, sometimes contain no fecal material. They consist entirely of a clear mucoid discharge resembling egg-albumin.

Recovery sets in, if not too large a dose is administered, within a few hours, usually 5 or 6, and is most frequently complete within 24 hours.

As one control measure, animals were inoculated intraperitoneally with sterile broth containing quantities of formalin (0.3%) and merthiolate (1:10,000) equivalent to those present in the filtrates prepared. 10 different animals were inoculated with quantities of from 1.0 to 5.0 c.c. In no case was there a suggestion of the symptoms characteristic of the syndrome produced by the enterotoxin present in the filtrate of strain "B".

As a further control measure, filtrates of strain "Wood 46", prepared under conditions identical with those of strain "B", were inoculated into cats on a number of occasions; doses varied from 1.0 to 5.0 c.c. The animals remained normal in every case. Filtrates of "Wood 46" have been ingested by human volunteers in relatively large amounts and have occasioned no upset.

In all, some 50 individual experiments have been performed on cats with filtrate "B", using intraperitoneal inoculation. Doses varying from 0.4 to 3.0 c.c. were employed, and in every case, symptoms of gastro-intestinal distress have been present in greater or lesser intensity, depending

upon the size of the dose and the degree of active immunity acquired by the animal due to previous injections.

The filtrate "B" producing gastro-enteritis by both enteral and parenteral administration in cats was inoculated into a dog, 3 months of age. 3.0 c.c. were injected intraperitoneally. Vomiting began 5 minutes after inoculation and continued at intervals for over 3 hours; several fluid stools were passed within one hour of inoculation. The animal appeared dazed and giddy, and was too weak to stand or sit up. The following day it walked unsteadily and refused food; recovery seemed complete however, within 48-60 hours. The sensitivity of dogs to the enterotoxin has been demonstrated on several occasions. In every case the symptoms are identical with those produced in cats by inoculation of the gastro-intestinal irritant.

During the course of investigation, certain observations were recorded that tended to confirm statements made by other workers, as well as by the author earlier in this paper. Jordan and Burrows (21), Woolpert and Dack (16) and later Dolman (22) produced evidence to show that the enterotoxic principle was not identical with the hemolysins, lethal or other recognized toxic products of staphylococci. The method employed in this investigation of preparing enterotoxic filtrates devoid of these substances provides further data in support of this contention. The author wishes to present additional experimental evidence in favor of the fact that the enterotoxin is a separate toxic product of staphylococcus.

Despite the fact that:

(a) the hemolysins α and β were no longer detectable in filtrates of "B" employed in the experiments;

(b) no skin reaction could be demonstrated;

(c) 1.0 c.c. quantities inoculated into mice, and 5 to 10 c.c. quantities into guinea-pigs occasioned no harmful reaction; some cats died following parenteral inoculation of the enterotoxic filtrates.

Several protocols are presented to illustrate the phenomenon.

Protocol 12.

Cat No.1   1-2 months old. Weight 600 g.

2.0 c.c. filtrate "B" were inoculated intraperitoneally.
Time: 11:10 A.M.
30 Mins. A thin watery stool was passed.
1 Hr. 5 Mins. Violent vomiting occurred. Vomiting and diarrhoea recurred at intervals for several hours. The animal was weak, dazed, and suffered acute abdominal distress. Death occurred at 12:15 the following day, 25 hours after inoculation. Just before death the animal appeared markedly emaciated and in a state of extreme prostration. Mucus was discharged involuntarily from the anus at frequent intervals. The animal died during a spasm of convulsive peristalsis involving both the oesophagus and intestinal tract.

Autopsy: Emaciation was very marked. The peritoneum and site of inoculation showed nothing abnormal. The intestines were completely contracted, blanched and very firm in consistency. Internal examination of the gut revealed an excessive secretion of a thick yellow mucus throughout the entire length. The stomach contained a large quantity of gas. The gall bladder was enormously engorged, and diffusion of bile pigments stained the viscera over a wide area. The urinary bladder was completely contracted and firm. No further macroscopic abnormalities were apparent.

Proctocol 13.

Cat No.2 1 month old. Weight 350 g.
July 2, 1936. 0.1 c.c. of filtrate "B" was administered intraperitoneally. There were no untoward symptoms except somnolence.

July 3, 1936. 0.2 c.c. of filtrate "B" were administered intra-
peritoneally. Some gastric discomfort and dizziness accompanied
by somnolence persisted for several hours following inoculation.
July 4, 1936. 0.3 c.c. of filtrate "B" were administered
intraperitoneally. There was general lassitude and discomfort
for several hours.
July 5, 1936. Diarrhoea was evident on July 5, and continued
for 8 days. Stools occurred frequently, often at 20 minute
intervals, and expulsion was involuntary in most instances.
Death occurred on July 13.

Autopsy: The tail and hind legs were soiled with mucus dis-
charged from the anus. The animal was pitifully emaciated;
the loss in weight over the 11 day period exceeded 40%. The
peritoneum was normal; there was no reaction at the site of
inoculation. Both large and small intestines were contracted,
hard, blanched, and, on internal examination, showed excessive
secretion of mucus throughout their entire length. The stomach
contained a large quantity of mucuous secretion. The gall
bladder was engorged; diffusion of bile pigments stained the
abdominal viscera over a wide area. The urinary bladder
was completely contracted and firm. There were no further
macroscopic abnormalities.

These examples are typical of some 20 reactions observed

when filtrate "B" has been inoculated intraperitoneally into

cats. In all cases, the only constant abnormalities noted on

autopsy have been those presented in the above protocols. In

no instance were the signs of general vascular engorgement and

petechial hemmorhages, characteristic of rapid death from the

effects of staphylococcus exotoxins, present to any observable

degree.

This phenomenom has also been produced by repeated

subcutaneous inoculation of the filtrate. Diarrhoea occurss

about 24 hours after the first dose, and continues with inc-

reasing severity until death. Vomiting however, has not been

observed on subcutaneous administration of enterotoxic filtrates.

A large dose of filtrate "B", or the frequent

administration of smaller doses, produces an exaggerated form

of the usual temporary gastro-intestinal upset. The absence of the hemolysins, dermo-necrotic and lethal substances in the original filtrate employed, combined with the fact that none of the characteristic post-mortem symptoms of acute death from potent exotoxic filtrates as described by Dolman (25) were observed, strongly indicates that the gastro-intestinal irritant is a substance <u>entirely separate from any of the recognized toxic products of staphylococci</u>.

The heat stability of staphylococcus enterotoxin has been recognized for several years. Jordan, et al. (20) using human subjects as test animals stated; "The toxic substance present in staphylococcus filtrates, causing gastro-intestinal derangement, is not completely destroyed by exposure for 30 minutes to the temperature of boiling water. Some diminution in toxic power may, however, possibly be caused by heating even at temperatures below 100° C."

Filtrates of strain "B" heated at 100° C. for 30 minutes have, on intraperitoneal inoculation in cats, produced the gastro-intestinal syndrome characteristic of the enterotoxin. Some diminution in toxic power is evidenced by the fact that larger doses of the same filtrate are required to produce typical symptoms after heating at 100° C. for 30 minutes, than are necessary after heating at 100° C. for 10, 15, or 20 minutes. The toxin is, however, not completely destroyed by heating at 100° C. for 30 minutes, when assayed by the "kitten-test".

Earlier publications noted that the enterotoxin is stable to storage at low temperatures. In this laboratory preparations from strain "B" containing 0.3% formalin, maintained at refrigerator temperatures, show little diminution in toxic power after 5 months. Incubation at 37° C. in the presence of 0.3% formalin for 78 days did not destroy the enterotoxin although some detoxification took place.

Barber (2) in 1914 and Dack et al. (23) in 1928 reported that no perceptible degree of immunity or tolerance could be acquired by repeated ingestion of enterotoxic filtrates. Woolpert and Dack (16) stated in a later publication that a degree of active immunity against the enterotoxin could be induced by repeated parenteral inoculation, but all attempts at passive immunity failed.

Working with cats in this laboratory, it came to one's attention that, after a number of injections, an animal was able to receive with impunity several times the dose of filtrate "B" that previously caused symptoms of acute gastroenteritis. During the course of investigation this phenomenom was consistently in evidence. The active immunity described by earlier workers could, then, be induced by repeated parenteral inoculation of filtrate "B".

Further investigations were carried out to determine whether the serum of those animals acquiring active immunity in this manner would neutralize the enterotoxin of filtrate "B". Serum was obtained from actively immunized cats and incubated with filtrate "B" at 37° C. for $1\frac{1}{2}$ hours. The mixture

was inoculated intraperitoneally into cats.

Protocol 14.

Cat No.1 1.5 c.c. filtrate "B" + 2.0 c.c. immune serum incubated at 37° C. for 1½ hours were inoculated intraperitoneally. The animal remained normal, played, and ate heartily whenever food was offered. No symptoms of gastro-intestinal distress were present.

Cat No.2 1.5 c.c filtrate "B" + 2.0 c.c. saline incubated at 37° C. for 1½ hours were inoculated intraperitoneally. The animal showed symptoms of acute gastro-intestinal upset. Diarrhoea continued for some 5-7 hours.

Cat No.3 1.5 c.c. of saline + 2.0 c.c. immune serum incubated at 37° C. for 1½ hours were inoculated intraperitoneally. The animal remained normal.

Protocol 15.

Cat No.   1.0 c.c. filtrate "B" + 1.5 c.c. "immune serum" incubated at 37° C. for 1½ hours were inoculated intraperitoneally. The animal remained normal.

Cat No.   the following day - 0.5 c.c. filtrate "B" were inoculated intraperitoneally. Vomiting and diarrhoea occurred. This animal, apparently sensitive to 0.5 c.c. of filtrate, received with impunity three times that amount mixed with immune serum.

Rabbits were given repeated parenteral inoculations of filtrate "B", and the serum was tested for immune substances against hemolysins. In this experiment, a filtrate of strain "B" containing all of the toxic substances was used. It was not detoxified with formalin. Sufficient serum was added to the toxin to neutralize the hemolysins. The mixture was incubated overnight at 37° C., the precipitate removed and the supernatant tested on cats.

Protocol 16.

Cat No.1 1.5 c.c. toxin "B" heated at 100° C. for 10 minutes (to destroy the α and β and lethal toxins,) were inoculated intraperitoneally. Severe gastro-intestinal symptoms appeared 10 minutes after inoculation. Vomiting and diarrhoea recurred at intervals for some time.

Cat No.2 4.2 c.c. of toxin-antitoxin mixture (3.0 c.c. toxin "B", 1.2 c.c. anti serum) were inoculated intraperitoneally. The animal remained normal.

Cat No.3 1.5 c.c. toxin "B" heated at 100° C. for 10 minutes (to destroy the ∝ , β hemolysins and lethal toxins,) plus 1.5 c.c. normal rabbit serum incubated overnight at 37° C. were inoculated intraperitoneally. Violent vomiting and diarrhoea appeared in 10 minutes and recurred frequently over a period of 2-3-hours. The stool was a clear mucoid secretion containing no fecal material.

An antiserum was prepared by immunizing a rabbit with filtrates of strain "Wood 46", containing all of the toxic products of staphylococcus except the enterotoxin. The antiserum was capable of neutralizing in vitro the ∝ and β hemolysins.

Protocol 16 (cont'd).

Cat No.4 1.5 c.c. toxin "B" 3.0 c.c. antiserum "W" (sufficient to neutralize the ∝ and β hemolysins) incubated at 37° C. overnight, were inoculated intraperitoneally. Within 10 minutes, violent vomiting occurred, followed by diarrhoea. The animal was ill throughout the day. Death occurred the following day, and autopsy revealed only the symptoms typical of those produced by the enterotoxin.

It would seem evident that antisera prepared against filtrate "B" are capable of neutralizing in vitro, the gastro-intestinal irritant contained in that filtrate.

Cat No.2 (protocol 16), received with impunity twice the dose that produced violent gastro-enteritis in animals 1, 3 and 4. The substance that neutralizes the enterotoxin is not present in normal rabbit serum, (cat 3) nor in antisera prepared against filtrates containing all of the known toxic products of staphylococcus except the enterotoxic principle (cat 4).

The investigations recorded up to this point have been
carried out on filtrates of a strain tested by human volunteers,
and known to produce a powerful enterotoxin. The sensitivity
of cats and dogs to the gastro-intestinal irritant has been uti-
lized in examining strains from various sources.

1.   In an epidemic of food-poisoning reported by Dolman (26),
large numbers of hemolytic staphylococci were isolated from the
suspected pastry, custard filled "vanilla slices". Intraperi-
toneal inoculation in cats of suitably prepared filtrates from
this strain produced the unmistakeable symptoms of gastro-
enteritis described earlier in this paper.

2.   Hemolytic staphylococci isolated from a cream pie, suspected
of causing food-poisoning, yielded a powerful enterotoxin. 5.0 c.c.
of the filtrate inoculated intraperitoneally into a 3 months
old pup evoked violent gastro-enteritis. The dog vomited more
than 18 times within 30 minutes of inoculation.

3.   A golden staphylococcus reported to cause "caramel flavor"
in milk, produced a potent enterotoxin as well as high titres of
α and β hemolysins. The organism, obtained from the Department
of Dairying at the University of British Columbia, was class-
ified as "Tetracoccus liquefaciens" (Orla-Jensen). In 10 exper-
iments with this strain positive results were obtained in every
case with doses of 1.0 to 3.0 c.c., and death occurred in 3
instances, from doses as low as 1.5 c.c. A similar instance
has been described by Tanner and Ramsey (5).

4.   An albus strain, isolated from raw milk, produced high titres
of α and β hemolysins as well as a powerful gastro-intestinal

irritant. 8 experiments were carried out with appropriately prepared filtrates. Doses as low as 0.5 c.c. produced positive results, and death occurred in 3 instances from doses as low as 2.0 c.c.

5. From a total of 8 strains picked at random from raw milk bottled for local distribution, 6 produced a powerful enterotoxin when assayed by the kitten test.

Discussion.

The public health issues involved in the study of staphylococcal gastro-enteritis cannot be readily overlooked. Since 1928 there have been reported some 47 outbreaks of staphylococcus food-poisoning; 40 of these involved more than 1400 persons of both sexes and all age groups. The failure to recognize the signifigance of the micro-organism in such outbreaks prior to 1928 has been attributed by some authors partly to the influence exerted by the exhaustive studies of Savage (28) and Ekkeles and Standfuss (29) on the Salmonella group; and while in certain instances staphylococci were known to be present in foods incriminated epidemiologically, their presence was not reported by the laboratory. Furthermore, a transient disability such as that caused by the enterotoxin would only too frequently be diagnosed by the layman as "intestinal flu" or "summer diarrhoea" one would expect relatively few such cases to be reported to authorities for full investigation. These considerations, combined with the fact that the texture, appearance and taste of food heavily contaminated by staphylococci remain unaltered, would lead one to believe that staphylococcal gastro-enteritis is more common than reports indicate.

The high percentage of outbreaks attributed to milk and milk products is a public health issue of special signifigance. It has been demonstrated that contamination arising from staphylococcal infections of the udder (2, 8) and perhaps staphylococci in milk obtained from apparently healthy cows may play a part in

outbreaks of food-poisoning (6,8). Inasmuch as this source of
contamination cannot be readily controlled by any method of pro-
duction, the only effective preventative measure would be univer-
sal pasteurization. Where raw milk is distributed there is the
additional hazard of droplet infection by staphylococci from
those handling the milk during production; and since milk seems
to provide an excellent medium for the rapid multiplication of
the micro-organisms, adequate cooling and refrigeration methods
should be enforced.

The frequent incrimination of cream pies and custard-filled
pastries presents a problem of interest and importance. Meyer (17)
contends that droplet infection, and the transmission of staphy-
lococci from superficial lesions on the hands of employees are
the most common methods of contaminating these products. Slow
cooling in large open kettles at room temperature prior to
filling pastry shells enhances the risk of contamination, and
provides admirable temperature conditions for rapid multiplication.
By virtue of the large batches of filler prepared in bakeries,
the number of persons involved in outbreaks from this source
is frequently high. (Table1.)

Meyer(17) reports an increased incidence in the Southern
United States during the warm summer months. Thorough cooking
and prompt refrigeration of the fillers and finished products,
as well as daily sterilization of utensils have been advocated.
Some health authorities in that region have, during the warmer
months, restricted the sale and distribution of cream-filled

bakery goods to bakeries with properly refrigerated storage facilities and display cases. An unofficial report indicates that enforcement of the latter measure has actually reduced the .incidence of staphylococcal gastro-enteritis in the area concerned.

Methods of preparation could be discussed at great lenth, but it should suffice to stress:

(a) pasteurization of milk and milk products,

(b) frequent sterilization of bakery utensils, and cleanliness in methods of production,

(c) adequate refrigeration facilities, and,

(d) the strictest observance of the ordinary rules of hygiène by those handling food for human consumption.

While there is as yet insufficient experimental evidence available from which to draw definite conclusions regarding the mode of action of the enterotoxin on the animal system, there are certain interesting points which might tend to throw some light on the matter. Despite the fact that the more obvious reactions produced by the toxin are clinical signs of a gastro-enteritis, post-mortem examination of animals dying from the effects of large intraperitoneal inoculations of filtrates containing only the enterotoxic principle does not reveal signs of acute intestinal inflammation. There is an excessive secretion of mucus; but this condition does not appear to result from an enteritis in the pathological sense. Furthermore, the rapid onset of the symptoms, frequently within 5 minutes of intraperitoneal inoculation, would scarcely be due to an inflammatory

process arising from direct irritation of the intestinal mucosa. The absence of the signs of local inflammation at the point of inoculation or in the peritoneal cavity excludes the possibility of extensive damage through contact between the toxin and exposed tissues.

The mode of action would appear to be through the nervous system. This would necessitate absorption of the toxic substance, and transportation by the blood to the nerve centres attacked. The possibility that this may actually occur is supported by the variable length of the latent period, and by the severity of the symptoms varying with the route of administration. If the substance be ingested with food, the latent period is $1\frac{1}{2}$-3 hours, which may be taken to correspond with the period required for digestion and subsequent absorption of the ingested material. In intraperitoneal inoculation the latent period is shortened to as little as 5-10 minutes, due to more rapid absorption by that route. The reaction is more severe in intraperitoneal than in oral administration, and it is probable that the slower absorption in the latter case, combined with the fact that much of the toxic material may be discharged in vomitus and feces, only a small part of the ingested dose is actually absorbed. This factor is eliminated by parenteral administration, and intraperitoneal inoculation evokes the most rapid, violent and persistent symptoms. In subcutaneous inoculation symptoms are less severe, and appear after 18-24 hours. The onset of symptoms would seem to demand a certain prior concentration of

toxin in the blood stream, the latent period being the time required for that concentration to be reached, and the length of the latent period depending upon the rate of absorption. The nerve centres attacked by the enterotoxin are not known. In post-mortem examination, the urinary bladder has been consistently found in a state of complete contraction, which would discount the possibility that peripheral nerve endings in the gastro-intestinal tract alone have been affected. Moreover, that fact that the vomiting and diarrhoea which follow the administration of the enterotoxin to laboratory animals are usually associated with unsteadiness and apparent vertigo, strongly suggests that the substance concerned may act upon adjacent centres in the mid-brain whose excitation results in the syndrome recorded.

The discovery of active immunization methods with staphy-lococcus toxoid necessitated the establishment of arbitrary standards for innocuity and potency to which preparations for clinical use could be referred before release from the laboratory (24). Since the inclusion in the toxoids of filtrates containing enterotoxin might give rise to unpleasant reactions, precautions must be observed to prevent their being used in preparations for human immunization. The suggestion has already been made (30) that an additional standard for innocuity should be appended to those recommended by Dolman and Kitching (24), viz.; "3.0 c.c. of the toxoid , inoculated intraperitoneally into a normal kitten should occasion no deleterious symptom".

But strains of staphylococci isolated from human lesions and subsequently found able to produce enterotoxin, may conceivably owe their pathogenicity in part to this latter property. The reaction of the host to continued absorption of small quantities of enterotoxin, such as may occur in acute staphylococcal infections, is not definitely known; but in the light of our knowledge of the deleterious effect upon cats under certain circumstances of repeated parenteral inoculation, it is possible that the enterotoxin might play an important rôle in human staphylococcal infections. Since the enterotoxin is antigenic it would seem desirable that efforts should be made to render the antigen innocuous to humans so that it might be incorporated with other staphylococcal antigens in materials distributed for the active immunization of man.

Further work is desirable on the problem of the antigenic properties and relationships of the enterotoxin, and methods of assay of anti-enterotoxic sera require investigation. In the meanwhile, filtrates of strains producing enterotoxin might to advantage be included in the pooled antigens used for the immunization of horses in the preparation of staphylococcus antitoxic sera.

Summary.

1. Certain strains of staphylococcus produce a gastro-intest-
inal poison in conjunction with various other toxic substances.
This enterotoxin may be separated from these lethal toxins,
hemolysins and necrotoxin by formalinization at 37° C. until the
α and β hemolysins are no longer detectable.

2. The intraperitoneal inoculation of small amounts of these
formalinized filtrates in cats and dogs provides a test suffic-
iently sensitive for demonstration of the presence of the ent-
erotoxin by evoking unmistakeable symptoms similar to those
observed in humans suffering from staphylococcal food-poisoning.
Guinea-pigs, rabbits and mice are not sensitive to oral or par-
enteral administration of the filtrates.

3. The gastro-intestinal syndrome in parenteral inoculation is
an intensified form of that produced by oral administration;
excessive dosage may prove fatal. Post-mortem changes differ
from those present in animals dying from the effects of the
potent exotoxins of staphylococcus.

4. The substance producing gastro-intestinal derangement in
cats posess properties similar to those ascribed by otherwork-
ers to the enterotoxin affecting humans. Thus, it is distinct
from the hemolytic, demro-necrotic or lethal substances; heat-
ing at 100°C. for 30 minutes does not completely destroy the
enterotoxic principle; it is stable when stored at low temper-
atures; it will not distil; and a degree of active immunity can
be induced by repeated parenteral inoculation.

5.   The serum of an actively immunized animal apparently neut-
ralizes the enterotoxin in vitro.

6.   Strains of staphylococci from raw milk and pathological
lesions in humans may, under appropriate conditions, produce a
powerful enterotoxic substance.

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TABLE I.

Epidemics of Staphylococcus Food-poisoning. (Directly or indirectly reported.)
Note: This list makes no claim for completeness.

Date	Reported by	Journal	Location	No. Involved	Caused by	Laboratory report
1.1907	Owen	Physician and Surg., 1907, 29:289		19	dried beef	staph.
2.1914	Barber	Philippine Jour. Science, Sect.B., 1914,9:515	Philippines	several	milk vanilla ice cream cream pie	white "
3.1927-28	Arthur French	199:1145	Pasadena Cal.	?	chocolate cream pie	yellow "
4.1928	Nelson	New Eng. Med. Jour., 1928.	Boston Mass.	approx.150	Wedding cake	yellow "
5.1929	Jordan	J.A.M.A.,1930, 94:1648	Chicago, Ill.	20	Layer cake & cream puff (custard filled)	yellow "
6. 1929	Jordan	J.A.M.A.,1930, 94:1648	Chicago, Ill.			yellow "
7.1929	Dack, Cary, Woolpert and Wiggens	Jour. Prev. Med., 1930, 4:167	Chicago, Ill.	11	sponge cake, cream filler	yellow "
8.1930	Jordan	J.A.M.A. 1930, 4:167	Porto Rico	4	cheese	yellow "
9.1930	N.Y. state Dept. of Health	N.Y. Health News, Oct., 1930. Dec., 1930.	Long Island	4	cake	yellow "
10.1930	" " " " "		New Jersey	125	cream-puffs	"
11.1930	Jordan	J.A.M.A. 1930, 97:1704	Milwaukee	4	Devil's food cake	"
12.1931	Jordan & Hall	Jour. Prev. Med., 1931, 5:387	Panama	2	chicken gravy	"
13.1931	Jordan		Jersey City	16	layer cake	"
14.1931	Meyer		Calif.	43	chocolate eclairs	"
15.1931	Meyer		Calif.	42	head cheese sandwiches	"
16.1931	Meyer		Los Angeles Cal.	, 5	cocanut cream pie	"
17.1931	Meyer		Calif.	2	chocolate eclairs	"
18.1931	Ramsey & Tracy	Proc. of the Soc. for Exp. & Biol. med., 1931, 28:390		?	milk	Orange "
19.1932	Ramsey, Tanner	Am. Jour. Sc., 1932, 80-85:184	Calif.	20	milk	Orange "
20.1932	Meyer, Krueger		Calif.	20	kippers	"
21.1932	Meyer, Simpson		Washington	200	ground meat sandwiches	"
22.1932	Grieger, Gray, Meyer		San Francisco	35	chocolate eclairs	"
23.1932	Jordan, Burrows	Am. Jour. Hygiene, 1934, 20, No. 3:604-610	Milwaukee	54	custard filled pastries	"
24.1932	" "	" " " " " " "	Chicago	31	cream filled pastries	"
25.1932	" "	" " " " " " "	Milwaukee	7	custard filled coffee cake	"
26.1932	" "	" " " " " " "	Chicago	2	custard-filled doughnuts	"
27.1932	Buchanan, Ecker		Cleveland	several hundred	cream pie	"
28.1933	Jordan, Burrows,	Am. Jour. Hygiene, 1934, 20, No. 3:604-610	Winona, Minn.	several	chocolate eclairs	"
29.1933	McBurney	J.A.M.A., 1933, 100:1999	U. of Alabama	150	" "	yellow "

TABLE I Cont'd.

30	1933	Meyer		Oakland, Calif.	several	Cocoanut Cream Pie	Yellow Staph.
31	1933	Meyer & Wynns		Oakland, Calif.	6	Chocolate Eclairs	" "
32	1933	" " "		San Francisco "	4	Custard filled Cake	" "
33	1933	Meyer		Fresno, Calif.	6	Pastry & Pies	" "
34	1933	Meyer & Stone		Glendale, "	66	Bulk Ice Cream	" "
35	1933	Costa Mandray	Porto Rico Jour. Pub. H. & Trop. Med., 1933, 9:44	Porto Rico	?	Ham	Staph.
36	1934	Meyer		Calif.	8	Banana Cream Pie	Yellow Staph.
37	1934	"		San Francisco	1	Potato Salad	" "
38	1934	Meyer & Krueger		" "	5	Cream Puffs	Staph.
39	1934	Crabtree & Litterer	Am. J. P. H., 1934, 24:116	Tennessee	242	Milk	"
40	1935	Dack, Bowman & Harger	J. A. M. A., 1935, 105:1598		?	Tongue Sandwiches	"
41	1936	Shaughnessy & Grubb	J. Inf. Dis., 1936, 58:318		25	Cream Filling	"
42	1936	Dolman	Can. Pub. H. J., 1936, 27, 494	Vancouver, British Columbia	3	Custard Filled Pastry	"
43	1936	Owen			70	" " cake.	"
44	1936			Portland, Oregon	?	Cream Pie	"
45	1936	Meyer		Calif.	?	Pastry	"
46	1937	McCastline Thompson & Isaacs	Jour. Bact., 1937, 33:50		31	Ice-cream custard	"
47	1935	Corpening & Foxhall	Am. Jour. Pub. Health, 1935, 25:938		12	Custard filled cake	"

TABLE 2.

Food involved	Number of epidemics reported.	Approx. % of total	Number of persons involved.
Cream or Custard filled pastries and cakes including chocolate éclairs	21 7 28	57	600; in addition to several reports, number unspecified. 325 in addition to several reports, number unspecified.
Cake and pastry type unspecified	7	14.5	50 also reports, number unspecified.
Milk, cheese, ice cream	7	14.5	350 in addition to several large epidemics, numbers unspecified.
Meats, gravy	7	14.5	250 plus several large epidemics, numbers unspecified.

Note: In some cases several types of food from the same bakery were implicated.