it is hoped that the continued prosperity of this business may help make it possible for the Council of the Society to postpone still further the increase in dues which has been expected for the last few years.

GENERAL BACTERIOLOGY

G1. Bacteria in Travertine from the Yellowstone. CHAS. B. LIPMAN, University of California, Berkeley.

The work described in this paper concerns an extension of former studies of bacteria in rocks and in other old materials, such as adobe brick, old soils, etc., in which a search was made for microorganisms which have survived for long periods of time in a living condition. The method used for making the study on typical samples was to place a block of travertine from Terrace Mountain, which represents a long series of extinct calcareous hot springs, into molten paraffine at a temperature of about 200°C. for about 3½ minutes. The block was then broken open with sterile tools and pieces from the heart of the block were placed in a sterile moist chamber. The moist chamber was then transferred to a sterile inoculation chamber and pieces taken therefrom were flamed for 15 to 20 seconds in a flame with a temperature varying from 1300° to 1600°C. It was then instantly dropped into a sterile mortar and crushed. The crushed powder was then distributed into a variety of media and the bacteria were studied. Results of these studies show that there are filamentous bacteria of a remarkable and unique type in the travertine, which grow in travertine extract containing 1 per cent peptone and in travertine extract plus 1 per cent peptone plus soil extract. A variety of other media inoculated with the crushed travertine have not thus far yielded any growth. These bacteria are described, and photomicrographs accompany the paper.

G2. Stalked Bacteria, a New Order of Schizomycetes. ARTHUR T. HENRICI AND DELIA JOHNSON, University of Minnesota, Minneapolis.

A study of periphytic bacteria upon glass slides immersed in freshwater habitats shows the general occurrence of a group of bacteria, hitherto almost unknown, which secrete stalks by which they are attached to a firm substrate. Gallionella ferruginea Ehrenberg and Nevskia ramosa Famintzin are stalked bacteria previously described which are quite distinct from the forms we have observed. Pasteuria ramosa Metchnikoff, or a very similar form, has been rediscovered. We can add to these a variety of new forms, and propose the creation of a
new order, the Caulobacterales, to include all stalked bacteria. We suggest the division of this order into 4 families and 5 genera according to the following key:

A. Long axis of cell transverse to long axis of stalk; stalks dichotomously branched.
   I. Stalks lobose, composed of gum, forming zoögloea-like colonies.
      Family Nevskiaceae
      Genus Nevskia
   II. Stalks are twisted bands of ferric hydroxide.
      Family Gallionellaceae
      Genus Gallionella

B. Long axis of cell coincides with axis of stalk; stalks slender, filamentous.
   I. Reproducing by transverse fission, stalks unbranched.
      Family Caulobacteriaceae
      Genus Caulobacter
   II. Reproducing by longitudinal fission and by budding; stalks often branched in whorls.
      Family Pasteuriaceae
      a. Stalks very short or lacking, cells sessile.
         Genus Pasteuria
      b. Stalks long and slender.
         Genus Blastocaulos

Several species have been grown in crude cultures in the dark. Their distribution indicates that they are heterotrophic aerobes. Their minute size and structureless cells indicate that they are bacteria. While resembling the Chlamydobacterales, they are neither filamentous nor ensheathed.

G3. The Relation of von Hibler's "Bacillus VI" to Bacillus carnis (Klein) and Its Differentiation from Other Microaerophilic Bacilli.
Nicholas D. Duffett and Ivan C. Hall, School of Medicine and Hospitals, Univ. of Colorado, Denver.

Four strains of von Hibler's "Bacillus VI" (1908), originally described as "Bacillus V" in 1899, were studied with reference to morphologic, cultural, pathogenic, and serologic properties. Three of these strains were received as pure cultures; one was contaminated by Corynebacterium lymphophilum (Torrey, emend, Bergey, et al.). All of the pure strains of von Hibler's "Bacillus VI" were shown to be identical and to constitute a distinct species of microaerophilic bacilli.

Von Hibler's "Bacillus VI" was identified with Bacillus carnis (Klein). The latter name should be used in preference to Hiblerillus sextus (Heller) and Bacillus lactiparicus (Lehmann and Süssmann) on the ground of priority.

The following observations of Zeissler relating to von Hibler's "Bacil-
lus VI” are hereby confirmed and restated in terms of Bacillus carnis:
(a) Bacillus carnis was a pathogenic, non-proteolytic, component of a
culture of “Bacillus enteritidis sporogenes” sent by Klein to von Hibler
and described by the latter as “Bacillus IV”; (b) Bacillus carnis grows
well under anaerobic and delicately under aerobic conditions with pro-
duction of gas in the ordinary liquid and semisolid mediums. Spores
are subterminal. Deep iron brain medium is not blackened. Gelatin
and Löffler’s coagulated serum are not liquefied. Gas is formed in milk,
but the medium is not coagulated. Glucose, galactose, levulose, su-
crose, lactose, maltose and salicin are fermented. Glycerol, mannitol,
dulcitol, rhamnose, and inulin are not fermented; (c) Bacillus carnis
produces a weak filterable exotoxin; (d) Highly specific agglutinins are
produced in rabbits as a result of subcutaneous injections of Bacillus
carnis cultures.
Bacillus carnis is hemolytic when grown aerobically or anaerobically
on plain or on glucose blood agar.
Agglutinins were produced with difficulty using young toxic cultures,
but easily using old non-toxic cultures.
Specific antiserums were produced which neutralized all of the cul-
tures of B. carnis, but not those of other microaerophilic and pathogenic
anaerobic bacilli.
Bacillus carnis differs from Bacillus histolyticus in its failure to blacken
iron brain, to produce tyrosine, and to liquefy gelatin, and in its ability
to ferment glucose and lactose; it differs from Bacillus tertius in having
subterminal spores, and being non-hemolytic and pathogenic, and in its
failure to ferment mannitol.

Thompson and J. C. Henthorne, Mayo Clinic, Rochester, Minn.
Gram negative, anaerobic, non spore-forming bacilli have been iso-
lated from various human lesions. Four strains resemble the descrip-
tion of Bacillus fragilis (Veillon and Zuber). Three of these strains are
similar, but the fourth strain differs from the others slightly in sugar
fermentation and agglutination reaction. The strains designated as
Bacteroides fragilis had been isolated from a mixed flora in a pelvic
abscess, a sacral sinus, and two suppurative appendices.
Another species of Bacteroides has readily been identified with the
description of Bacillus funduliformis. Bacteroides funduliformis had
been isolated in pure culture from four liver abscesses, a colostomy, and
from a lung abscess.
Bacteroides funduliformis and Bacteroides fragilis are easily differentiated by morphology, fermentation reactions, hemolysis of red cells and agglutination.

Another strictly anaerobic organism systematically studied in this group is a Gram negative bacillus which characteristically produces large amounts of gas on carbohydrate media.

**G5. The Bactericidal and Antiseptic Action of Preservatives Frequently Used in Biological Products, and the Effect of These Preservatives on the Potencies of the Products.** CAROLYN ROSENSTEIN AND IDA LEVIN, assisted by HATTIE LEVIN, Bureau of Laboratories of the Department of Health, City of New York.

A study of the bactericidal and antiseptic action of preservatives frequently used in biological products and the effect of these preservatives on the potencies of the products showed that no one of these preservatives alone completely controls all the types of contamination found in biological products. This is particularly true in the case of serums and antitoxic-globulins. It was further found that half quantities of phenol and merthiolate in combination (0.25 per cent phenol and 1–20,000 merthiolate) successively added to serums, antitoxic-globulins, and toxins destroyed diphtheroids, B. pyocyaneus, and staphylococci, whereas 0.5 per cent phenol did not destroy diphtheroids and 1–10,000 merthiolate did not destroy B. pyocyaneus, when these preservatives were used singly.

The bactericidal action of phenol and tricresol has a tendency to decrease after prolonged storage (ten months) while that of merthiolate and the combination of phenol and merthiolate was apparently unaffected. The potency of the products was not affected by 10 months of storage at 5°C. with phenol, tricresol, merthiolate, or the combination of phenol and merthiolate in the dilutions used in these tests.

It was found also that it is necessary to use both solid and liquid media in order to detect all the types of contamination which may be present in biological products.


Merphenyl nitrate (basic) is the double salt of phenylmercuric hydroxide and the normal phenylmercuric nitrate. It is soluble in water 1:1200. The phenylmercuric ion is believed to be the active com-
ponent. It differs from other mercurial compounds in that the mercuric ion is the cation. Since bacteria are usually negatively charged, this positive charge on the phenylmercuric ion may be responsible for its high inhibiting power.

Kolmer's bacteriostatic method was used to determine inhibition and with it a modification of the method, used by Birkhaug, in which a very small number of bacteria is used. By Kolmer's method E. coli was inhibited by a 1:420,000 concentration. Staph. aureus was inhibited by a 1:12,000,000 concentration. Using Birkhaug's method, similar to Kolmer's but using 200 to 2000 bacteria per cc., E. coli was inhibited by a 1:2,500,000 concentration. Staph. aureus was inhibited by a 1:307,000,000 concentration.

Determining the killing power of the substance, in the case of Staph. aureus, offered difficulties because of its high inhibiting power. This was not an apparent factor with E. coli. Shippen's modification of the Food and Drug Administration test was found necessary with Staph. aureus but even this method, though avoiding the gross error of confusing the inhibiting with the killing power, did not give dependable results. More satisfactory results were obtained by using a given dilution of the disinfectant and varying the time element, using plate counts in addition to the usual test in broth tubes. It is not believed, however, that the true killing concentration of such highly effective mercurials can be determined by any of the usual methods.


In view of the interest in industrial applications of the so-called "oligodynamic" effect of silver in the sterilization of water, the present investigation was undertaken to measure the relative germicidal effectiveness of dilute solutions of silver prepared from: (1) fused silver chloride, (2) silver nitrate, (3) silver acetate, (4) colloidal silver, prepared by passing an arc between 2 silver wire electrodes immersed in distilled water, and (5) a silver solution prepared by the Katadyn process from metallic silver using a dry cell as the source of current.

Two types of water were used in making up the test solutions: (1) sterilized river water having a slightly alkaline reaction, and (2) sterile distilled water to which 10 p.p.m. of CaCO₃ had been added and which had a reaction approximately neutral at the time of use.

Escherichia coli and Staphylococcus aureus were employed for evaluat-
ing the germicidal potency of the solutions tested. A known number of test organisms were added to the test solutions and plate counts made at various intervals. All tests were made at 20°C.

The results obtained on the 5 types of silver solutions indicate that a concentration of 0.10 to 0.15 p.p.m. of silver ion is sufficient to destroy *Escherichia coli*, when present to the extent of 250,000 per cc., within a period of 2½ hours. *Staphylococcus aureus* appeared to be only slightly more resistant to the action of the silver ion than *Escherichia coli*.


In all, 70 chemical compounds which have recognized fungicidal power or which have been recommended for the treatment of various mycoses were tested for ability to kill representatives of the group of yeast-like organisms producing vulvo-vaginitis.

The technique used was essentially that of a standard loop transfer method. An equal quantity of a broth suspension of young culture of the organisms was mixed with the chemical solution. The exposure time was 5, 15, and 30 minutes after which time a 3 mm. loop of the mixture was transferred to a Petri dish, and agar poured in at once. The chemicals tested were used in concentrations which may be tolerated by the vagina. Under these conditions, some of the chemicals were eliminated from further study.

Since the chemicals would be used in the presence of organic matter and cellular elements, a substitute condition for the vulva and vagina was attempted by tests repeated with the addition of blood serum and red blood cells. Under these conditions, all but a few of the compounds were ineffective. Those showing promise were metallic iodine, mercurophen, mercuric chloride, and chlorothymol.

A number of the compounds tested were known to be fungicides, and yet were very slightly or not at all effective against the yeast-like organisms used under these experimental conditions. The killing power of some of the chemicals employed was tested against members of the higher fungi: a common laboratory contaminant, *Monilia* sp., a plant pathogen, *Fusarium nivale*, and a dermatophyte. Differences were found in the susceptibility of these fungi to various reagents, but a greater number of chemicals were effective against these higher fungi than against the yeast-like organisms.

Pneumococci are especially susceptible to the action of certain unsaturated soaps, such as sodium oleate, linoleate, linolenate, and clupanodionate. These soaps are germicidal at 37°C in a concentration of 0.005 per cent, using the Reddish technique. Other unsaturated soaps, such as sodium abietate, chaulmoograte, undecylenate, alpha elaeostearate and beta elaeostearates, as well as hydroxylated and saturated soaps, are less effective. Bile salts and sodium salicylate require concentrations of 5 to 10 per cent to kill pneumococci in 10 minutes. Sulfate ester salts are pneumococcidal in concentrations of 0.01 per cent.

Streptococcus lactis is considerably more resistant to the action of soaps than the pneumococcus. In general, soaps which are germicidal against pneumococci are also germicidal against streptococci, but to a lesser degree. There appear to be exceptions to this general rule in the cases of sodium stearate, palmiate, alpha elaeostearate and beta elaeostearate, which are germicidal to pneumococci but not to streptococci.

Escherichia coli and Staphylococcus aureus were more resistant than the other two organisms tested. Of the compounds tested, only sodium 3,5-diiodo-salicylate and undecylenate were effective in concentrations of 1 per cent against these two organisms.

Detoxifying tests were made by mixing 1 cc. of a 2 per cent soap solution with an equal volume of normal saline containing 10, 30, 50, 70, or 100 M.L.D. of diphtheria toxin and allowing to stand 15 minutes at 25°C., after which 1 cc. of this mixture was injected subcutaneously into a 300-gram guinea pig, and survival or death of the animal noted. Ability to neutralize diphtheria toxin is a property which is common to all soaps. The degree of their effectiveness varies considerably with chemical constitution. Spatial configuration, as well as functional groups, affects the detoxifying power. One double bond increases markedly the detoxifying ability of a saturated soap, but the addition of further double bonds does not alter it. Hydroxyl groups and triple bonds affect the detoxifying power very slightly. The sodium compounds tested, listed in the descending order of effectiveness, are: chaulmoograte, ricinoleate, oleate, linoleate, linolenate, clupanodionate, alpha elaeostearate, lauryl sulfate, oleyl sulfate, ricinoleate, beta elaeostearate, ricinoleate, undecylenate, sativate, stearate, palmiate, myristate, laurate, trihydroxystearate, abietate, 3,5-diiodo-
salicylate, taurocholate, glycocholate, cholate dehydrocholate, salicylate, and gluconate.

The pH of all solutions was accurately controlled by means of the glass electrode.


Catalase exists in many cells in an inactive or an inhibited condition, and therefore escapes detection except under special circumstances. Observations on a suspension of young cells of *Proteus vulgaris* (Bergey) have shown that the apparent catalase content of the organisms may at times be greatly increased (10 x) by short pre-treatment with a heart muscle extract similar to the antigen of Kolmer.

A connection between catalase and cellular oxidation is shown by measurements on *Proteus* kept for 4 hours at different oxygen tensions. At high oxygen tensions the amount of active catalase is somewhat greater than at low oxygen tensions. The quantity of activable enzyme, however, is very much greater in the oxygenated cells (as determined by the increase in catalase activity after treatment with the muscle-extract activator). It seems probable that the higher rate of aerobic oxidation may stimulate the cells to the production of a potential supply of catalase greater than the actual needs of the organism, as evidenced by the supply of already active enzyme.

The inactive enzyme is distinctly of desmo type, for efforts to separate it from the live cells have failed. With cell death the inactive catalase disappears.

**G11. Studies on the Respiratory Mechanism of the Streptococci.** Michael A. Farrell, Department of Bacteriology, Yale University.

A collection of 22 representative strains of the genus *Streptococcus* has been studied with the object of determining the presence of respiratory enzymes, and gaining an insight into the respiratory mechanism of these organisms.

Catalase, cytochrome and indophenol-oxidase could not be demonstrated in the streptococci. The presence of a thermostable peroxidase was established. One hundred and one chemicals, including carbohydrates, amino, fatty, and other organic acids, were tested by Thunberg's technique (with certain necessary modifications) to determine the ability of streptococci to activate them as hydrogen donors. The
streptococci as a group possessed little or no activating power, except toward carbohydrates; most of the hemolytic organisms had no action on any of the sugars or higher alcohols.

The absence of cytochrome and indophenol-oxidase, corroborated by oxygen uptake studies and the ability of these organisms to grow in media containing a high concentration of KCN, indicate that a hemin respiratory mechanism of the Warburg type is absent from the streptococci.

A definite relationship between the thermostable peroxidase and the thermolabile dehydrase of streptococci was shown; this relationship is suggested as constituting an important part of the respiratory mechanism of this group of bacteria.


In a previous report by one of the authors, mucoid, smooth, and rough phases of Streptococcus hemolyticus were described. In the present report evidence is presented to show that these variant forms show a close analogy with the corresponding variant forms of pneumococcus and other bacterial species. Various stages in the S → R and R → S transformations are described and on the basis of these observations the suggestion is made that these variations are cyclical in character.

Further studies on the mucoid phase of Streptococcus hemolyticus are reported. These studies show that virulent hemolytic streptococci usually produce mucoid colonies but that organisms in the mucoid phase may or may not be virulent for white mice. The importance of the appropriate culture phases to be employed in subsequent studies with Streptococcus hemolyticus and other bacteria is emphasized. Finally, it is pointed out that any attempt to classify the hemolytic streptococci should be based on a study of analogous phases of development.


In dissociation studies of yeast Saccharomyces aceris-sacchari Fabian and Hall and Pichia alcoholophila Klöcker were used. The first named yeast normally produces a bottom growth in malt extract broth and smooth glistening colonies on malt extract agar. When grown and
transferred daily in 0.1 per cent LiCl yeast extract broth, there was a gradual transformation from the normal more or less round cells, 3.5 to 7 microns, into two types of cells. One type of cell was round, 6 to 10 microns, the other elongated, 10 to 16 microns. These cells contained large vacuoles around the periphery of which were located one to 15 or more fat globules, or oil drops. The colonies from both of these types of cells were extremely rough. When cells from these colonies were grown in malt extract broth for one or two days, they produced a rough spreading colony with radial folds which grew over the entire surface of the petri dish in a few days. The cells of this colony were round and measured 6 to 10 microns. When the amount of LiCl was increased to 0.25 per cent and cells from the rough colony were grown in it, extremely long cells, 16 to 22 microns, were produced. The colony produced by these cells in malt extract broth was a rough spreading colony without radial folds. All rough forms returned to the smooth form after several rapid transfers in malt extract broth.

Pichia alcoholophila Klöcker is normally a smooth yeast with growth at the bottom in liquid media. It produces smooth and glistening colonies. The normal smooth cells average about 6 microns in length, are round to slightly elongated in shape, and frequently produce pseudomycelia. A stock culture showing a wrinkled growth typical of rough yeasts was found to contain cells with small oil drops. An attempt was made to dissociate this yeast by growing it in 0.1 per cent LiCl broth, but the yeast could not tolerate it. After a few transfers in yeast extract broth, which has been found in our work to be a mildly dissociating agent, the cells developed large oil drops and vacuoles and the colonies became extremely rugose. Young colonies were composed of cells the same size as the normal smooth cells. Vacuoles were not prominent and oil drops were small or lacking. When the colonies were about six days old, a hanging drop showed many small bodies about 0.7 micron in diameter present along with the cells. The nature of these bodies has not been determined. When the colonies were about 7 or 8 days old, the cells became much larger, averaging 10 microns with vacuoles occupying most of the interior. Metachromatic granules could also be seen moving about inside the vacuoles and the oil drops became very prominent. Pseudomycelial formation was also common at this stage.

When giant colonies of this R form are made on malt extract agar and incubated for 10 to 15 days, secondary smooth colonies appear at several points on the periphery. If these are subplated, they produce
smooth colonies, the cells of which have the same appearance as the normal S cells except for the presence of small oil drops. The oil drops disappear only after many rapid transfers in malt extract broth. The R form when once produced remains stable in yeast extract broth.


The common occurrence of metabolic "mutation" in the genus Bacterium, the frequency of B. coli mutabile (Massini) in the intestinal tract of man, and complications brought about by B. coli mutabile in the recognition of B. paratyphosus in the feces of patients and carriers, in food poisoning outbreaks, and in the evaluation of potability of water supplies, are emphasized.

The ultimate criterion of lactose "mutation" is the repeated isolation of rapid lactose fermenters from a parent type which perpetuates both itself and the "mutant" indefinitely, but this criterion is usually preceded by observations of slow fermentation of lactose and of multiple papillae or secondary fermenting colonies on lactose agar media, such, for example, as eosin methylene blue lactose agar. The "mutant" type rarely, if ever when pure, reverts to the parent type, and, as a rule, is indistinguishable from it morphologically, culturally, and serologically, except by its more rapid fermentation of lactose, and by its inability to form characteristic multiple papillae or secondary colonies. Secondary colonies of a different type from those seen in the parent culture on lactose media may occur, however, both in the parent type on non-lactose media, and in the "mutant" on various solid media on long incubation.

The same criteria of metabolic "mutation" are known to apply to certain forms in relation to other carbohydrates and are probably capable of general application.

Data and illustrations are presented to show the simultaneous but independent occurrence of lactose mutation and colonial dissociation in B. coli mutabile, B. cloacae, B. dysenteriae dispers, and B. dysenteriae Sonne.

G15. Bacterial Variation: An Inquiry into the Underlying Principles Governing the Cell Morphology of Bacillus megatherium. Leo F. Rettinger and Hazel B. Gillespie, Yale University, New Haven, Conn.
In the present investigation we have attempted to throw some light on the significance of the variant cell forms so frequently seen in bacterial cultures, by inquiring into the fundamental principles which govern their existence. We have followed, under the microscope, the formation and continued development of Bacillus megatherium cells (both variant and ordinary rod forms) growing in microcultures on hanging blocks of agar, and have observed the effect of varying environmental conditions upon cell morphology in this species.

Cell form appears to depend largely upon the environment with which the growing organism has to contend. Slight changes in environment produce striking changes in cell shape. Cells developing on agar which is as yet unaffected by inhibitory metabolic influences occur as “normal” rods, no matter what the age of the culture may be; i.e., the periphery of a 20-day old agar colony is made up of young, hyaline rods, if the colony is far removed from the metabolic influences of surrounding colonies (well-isolated). On the other hand, the peripheral cells of even young colonies vary strikingly on crowded blocks where they are subjected to the diffusing “growth” influences from near neighbors. The type of cell produced seems to depend directly upon the degree of crowding.

Partial oxygen starvation is one of the important factors associated with crowded conditions. That it stimulates at least certain types of cell variation has been clearly demonstrated. When the oxygen supply of a culture is so limited, either by the metabolic influence of associated growth or by mechanical means, as to prevent the development of “normal” rods, globular and streptococcoid forms appear. Such cells are able to withstand conditions of moderately severe anaerobiosis for relatively long periods. They return rapidly to the “normal” rod form when oxygen is again supplied chemically or mechanically.

The factors responsible for variation are definitely unfavorable to growth, and if they accumulate very rapidly in a culture, all development ceases before variation can occur. In other words, variation is possible only where unfavorable and favorable environmental conditions are so balanced as to permit slow growth in the face of adverse circumstances. Variant cell forms do not appear in colonies uniformly distributed throughout the cell mass, but arise in definitely localized areas often in successive rings at colony peripheries. We have observed the growth of clubbed, branched, wedge-shaped, filamentous and globular cells. These variant forms seem to be better able to resist the unfavorable conditions that call them forth than are “normal” rods and, there-
fore, may be thought of as adaptations of the organism which favor the
continuation of the species. No evidence has presented itself to us,
however, which would indicate that they have any significance as "life
cycle" forms.

1G6. The Relation between the Oxidation-Reduction Potential of the
Medium and the Dissociation of an Acid-fast Organism. Janet
McCarter and E. G. Hastings, University of Wisconsin, Madison.

The cultures used were single cell strains of the rough and smooth
types of a non-pathogenic acid-fast organism isolated at the University
of Wisconsin.

Oxidation-reduction potential measurements, made electrometrically,
on dissociating smooth cultures in liquid media, and the dissociation of
rough and smooth cultures in oxidized and reduced media, show that
oxidized media favor the dissociation of the rough to the smooth type,
and reduced media favor the dissociation of the smooth to the rough
type.

Plate cultures of dissociating smooth cultures in liquid media were
made at intervals. A study of the colonies formed gave evidence that
the transition from a smooth to a rough type is not abrupt, but that
there are several intermediary types between a very smooth and a very
rough type. These various intermediary types dissociate more readily
in liquid media than does an extreme rough or an extreme smooth type.

G17. The Use of Semi-solid Agar for the Detection of Bacterial Motility.
Ralph P. Tittsler and Leslie A. Sandholzer, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

A study of semi-solid agar for detecting motility has been made with
1028 cultures and the results compared with those of the usual hanging-
drop technique. The agar (nutrient broth plus 0.5 per cent agar) was
inoculated by stabbing and incubated at 37°C. for 6 days. Motility
was manifested macroscopically by a diffuse zone of growth spreading
from the line of inoculation. Hanging-drop preparations were made
from broth cultures incubated at 37°C. for from 12 to 24 hours.

In hanging-drops, 500 cultures were motile. In agar, after 1 day,
497 were motile and 2 were questionable; after 2 days, 520 (including
all cultures motile in hanging-drops) were motile and 6 were question-
able. Further incubation failed to alter the questionable reactions in
these 6 cultures. Thus, after 1 day, agar yielded results essentially identical with hanging-drops but after 2 days it elicited 4 per cent more positive tests. A few agar cultures, chiefly members of the Escherichia genus, after 2 days showed evidence of motility by the formation of nodular outgrowths along the stab. Isolations from these nodules and from the non-diffused stab yielded respectively motile and non-motile strains identical in other cultural characteristics. Thus motile variants have been obtained from some cultures which were non-motile in hanging-drops. The semi-solid agar technique is particularly advantageous in teaching schedules and routine testing, because the results are accumulative and macroscopic.

G18. Studies on the Voges-Proskauer Test. W. Dorner and Esther Hellinger, Swiss Dairy Research Institute, Liebefeld-Bern, Switzerland. (Read by title.)

A comparative study was made on the Voges-Proskauer, Werkman, Leifson and O'Meara methods of detecting the production of acetyl-methyl-carbinol by microorganisms in a dextrose-peptone medium. Likewise the choice of one or more peptones from a range of peptones on the market for the special preparation of the medium was investigated.

In testing the acetyl-methyl-carbinol reaction with organisms of the aerogenes group the choice of a special peptone was not of special consequence. All the methods for conducting the test gave a positive result but certain methods produced a stronger color reaction than others. With other organisms, as for instance Streptococcus cremoris, a selection of medium and method of testing was found to be necessary so as not to overlook the possibility of obtaining a positive result.

A peptone broth medium is recommended. This should contain 0.7 per cent dextrose, 1.0 per cent peptone with meat broth as a basis. The following peptones may be used: Peptone Witte, Difco's Bactopeptone and Neo peptone, Fairchild's peptone or Merck's peptone for bacteriological purposes.

A combination of the methods of Werkman and O'Meara proved to give clearer and more positive results than either method alone. The method recommended is to take 1 cc. of the culture in a wide test tube, add 1 drop of 1 per cent creatine, shake; 1 drop of 2 per cent FeCl₃, shake well; add 1 cc. 20 per cent NaOH and shake. Place at about 37°C. but refrain from shaking the tube in the early stages of the test so as to observe sooner and more clearly the positive result given by an
eosin-pink to bright red coloration forming at the surface and diffusing downwards through the medium.


An attempt was made to separate from ordinary veal infusion the factors which are responsible for growth of some of the more fastidious bacteria. It was found that treatment of an ordinary veal infusion with charcoal resulted in the removal of certain unknown substances necessary for growth of these types and that charcoal was more effective for this purpose than other commonly used adsorbents. These unknown substances, or growth factors, after removal from an infusion in this manner, could be recovered from the charcoal by extraction with hot ethyl alcohol or hot acetone, although it is probable that this recovery was quite incomplete.

These extracts, after evaporation of the alcohol or acetone and subsequent solution of the residue in water, were effective in "activating" an infusion which had been previously treated with charcoal. They were also effective in rendering a standard beef extract-peptone broth more suitable for growth of certain exacting organisms.

The presence of essential growth factors in these extracts could also be shown by addition of the extracts to a synthetic medium. Small amounts of extract were sufficient to permit good growth of a number of organisms which refused to grow in the synthetic medium alone. Thus, several strains of Corynebacterium diphtheriae, Shigella dysenteriae, Eberthella typhi and Staphylococcus developed in the synthetic medium when the added material amounted to only 0.0006 to 0.00006 milligram of solids per cubic centimeter of synthetic medium. On the other hand, several strains of hemolytic streptococci, pneumococci, and Pasteurella refused to develop in the synthetic medium plus extract.

The growth factors dealt with here are believed to belong in the class of growth accessory substances. Their addition to synthetic media, even though in an impure state, offers a method for securing growth of certain of the more fastidious bacteria and for the study of various phases of metabolism of these types which can best be accomplished through the use of simple culture media. Additional work on other sources of growth promoting principles and on methods for their concentration and purification is now under way.
G20. II. Further Application of Semisolid Media in Cultivation and Identification of Sporulating Anaerobes. ROBB SPALDING SPRAY, Medical School, West Virginia University, Morgantown.

In 1933 the writer presented before the Society an outline of methods adapted to cultivation and identification of the sporulating anaerobes, which obviate all of the difficult and specialized technic commonly applied to this group of organisms. Since that time these principles have been extended over a larger number of species.

The tentative key, previously presented, has also been enlarged to cover these additional organisms. Many of these descriptions are based upon study of but a single strain, but such strains are of authentic origin. Others are based on study of from 5 to 25 strains. All tests have been repeated at least 3 times, and are regarded as reliable under conditions afforded by these methods.

In view of the fact that many descriptions are based upon study of such single strains, the key is still to be regarded as tentative only. We believe, however, that the significant reactions will be substantiated by further extension of the work. Results of further studies will be presented in subsequent reports.

G21. Stimulation of Bacterial Growth Rate by Methyl Germanic Oxide. PHILIP L. CARPENTER, MACDONALD FULTON AND C. A. STUART, Biological Laboratory, Brown University.

Methyl germanic oxide, 0.01 molar, in nutrient broth was found to have a stimulating effect upon the growth rate of organisms of many genera. Growth rate constants indicated that stimulation was greatest during the early logarithmic phase, but that the growth rate fell to zero earlier in stimulated cultures than in controls. A tendency to prolong lag was also observed. The action of methyl germanic oxide resembled in many respects the stimulating action of cations studied by Winslow and coworkers.

G22. Growth Curves of Escherichia coli and Salmonella schottmuelleri in Mixed Cultures. MACDONALD FULTON AND C. A. STUART, Biological Laboratory, Brown University.

The growth curves of pure and mixed cultures of Es. coli and Sa. schottmuelleri were studied in a synthetic medium. The chief difference between growth curves in the mixture and in pure cultures was in the length of the lag phase. Whichever species was numerically inferior at the outset underwent a prolonged lag in mixed culture, beginning
normal growth only after the other species had reached the period of declining numbers. Other differences between the growth curves of pure and mixed cultures were apparently due to pH changes in the medium. The reported antagonistic action of coli against schottmuelleri may be attributed to prolongation of the lag of the latter, not to a bactericidal effect.


The growth cycles of 4 common saprophytes—*Escherichia coli* (2 strains), *Aerobacter aerogenes*, *Serratia marcescens*, and *Chromobacterium violaceum*—were determined in nutrient broth at 22°, 27°, 32° and 37°C., the last temperature being the optimum for rate of reproduction of the various organisms. To insure a maximum rate and longer period of reproduction the inoculum was small (1000 to 2000 cells per milliliter) in every case. Enumeration of cells was by the plate count, using 5 plates of a suitable dilution of pooled, duplicate cultures. Counts were made every 2 hours for 48 hours.

At any one temperature, the rate of multiplication during the logarithmic phase of growth was nearly the same for the different organisms. An average generation time of 60 minutes at 22°, and 22 minutes at 37°C., was found. Calculation of the temperature quotient Q_{10°} for various temperature intervals showed that it decreased continuously with increase in temperature; that is, as the optimum was approached the increase in temperature had relatively less and less effect upon the rate of reproduction.

While the maximum rates of reproduction attained by the different organisms at the same temperature were nearly the same, the maximum numbers of cells finally reached in the cultures by different species at the same temperature showed wide variation; but for any one species the maxima reached were fairly consistent irrespective of temperature.

A constant relationship was found between the length of the logarithmic growth phase at any temperature and the rate of multiplication during that phase. At each of the temperatures used, the ratio of rate of reproduction (as generation time in minutes) to length of the logarithmic growth phase (in hours) was quite consistently 5:1.

Considered as typical population curves, these bacterial growth cycles were, as a rule, very asymmetric. While a "normal" S-shaped population curve is considered to be, theoretically at least, symmetrical, these
Curves all showed varying degrees of asymmetry in the upper part of the "S", which asymmetry was outside of the experimental errors of technique.

**G24. Chitinovorous Bacteria,—a Preliminary Survey. Anne G. Benton, Vassar College.**

By use of suitable enrichment and plating media, over 300 apparently pure cultures of chitinovorous bacteria have been isolated from a variety of sources, including water, soil, decaying animal matter and the alimentary tracts of birds, bats, frogs and fishes. At least 18 distinctly different types can be distinguished. Some of them seem to be fairly widespread in nature; others were isolated from but one source. None correspond to species now listed in Bergey's Manual, though a few may be related to recognized species. Atrichic, monotrichic and peritrichic rods are represented, as well as vibrios, spindle-shaped cells and pleomorphic forms. Among the latter types, a few seem to resemble cellulose bacteria from the soil, described by Winogradsky and others. The various morphological types exhibit interesting fermentative peculiarities, and are alike only in their ability to attack chitin. Some exhibit characteristic pigmentation. Their striking dissimilarity in gross and microscopic morphology, their differences in ability to attack various substrates, and their different types of fermentative attack on available substances, make it obvious that they should not be regarded as belonging to a single chitinovorous genus, but rather that they should be included in genera now recognized (or later to be defined) by morphological as well as cultural characteristics.

**G25. The Fermentation of Cellobiose by Bacteria. Ralph P. Tittsler and Leslie A. Sandholzer, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.**

The ability to ferment cellobiose has been determined for 755 cultures, representing approximately 50 species within the following genera: *Aerobacter, Escherichia, Proteus, Salmonella, Eberthella, Shigella, Alcaligenes, Serratia, Chromobacterium, Flavobacterium, Pseudomonas, Bacillus, Staphylococcus, Micrococcus* and *Sarcina*.

The results may be briefly stated as follows: acid and gas were produced by the *Aerobacter* cultures and most of the *Escherichia-Aerobacter* "intermediates," but not by typical members of the *Escherichia* group. All strains of *Eb. typhi, Sal. aertrycke* and *Sal. schottmuelleri* and 17 of 19 strains of *Sal. enteritidis* attacked this carbohydrate after from 4 to 10
days, whereas all strains of Sal. suipetifer, Sal. paratyphi, Sal. gallinarum, Sal. pullorum, Sal. morganii and the dysentery bacilli failed to do so within 14 days. Likewise, there were differences between certain other species, particularly those of the Proteus and Bacillus genera. In general, the cocci failed to ferment cellobiose. Since certain closely related groups or species differ in their ability to ferment cellobiose, this characteristic can serve a useful purpose in bacterial taxonomy.


Ernest A. Pribram and Louis Kotler, Department of Bacteriology and Preventive Medicine, Loyola University, Chicago.

In the first paper (J. Bact., 27: 24, 1934), differences have been reported between a beer yeast and a milk yeast concerning their capacity to combine with different carbohydrates, dextrose, galactose and lactose. Our present problem is to determine the influence of potassium and calcium chloride on the glucose combining power of yeast cells of different origin. The types of yeast cells were: Saccharomyces cerevisiae Hansen, type Frohberg, sedimentary beer yeast ("Frohberg"), "Saccharomyces" kephir Beijerinck, from kefir milk (the correct scientific name should be: Torulopsis beijerincki), Torulopsis rosea, isolated from air, and Cryptococcus hominis Busse, isolated from a case of thrush. The methods used for the determination of the capacity of yeast to combine with dextrose, were those published in the previous article.

To 1 cc. of the yeast suspension of 1000 cells per cubic millimeter, 0.02 cc. of 0.1 N solutions of the salts was added. All cultures were three days old, grown on dextrose agar. The combining power of the yeast for dextrose is based on its high content of phosphates (50–60 per cent of the inorganic ash), as stated by Wertheim, who found, that after adding glucose no glucose reaction (aldehyde reaction) was present in the thoroughly washed cells, unless the phosphorus-glucose ester had been hydrolysed by sulfuric acid. The results were as follows: The electrolytes KCl and CaCl₂ were in all experiments antagonistic in their action on glucose esterification by yeast cells. Their effect, however, was the opposite with different types of yeast. KCl decreased and CaCl₂ increased the esterification (loss of glucose in the suspension), if added to Frohberg yeast or to Torulopsis. KCl increased and CaCl₂ decreased the esterification, if added to kefir yeast or to Cryptococcus. It seems to be very likely that a certain ratio of different electrolytes,
especially of potassium to calcium ions is favorable for the esterification of glucose by yeast cells.

Analyses of the ash of Frohberg yeast and of a yeast from animal tissue (thrush) as found in the literature showed that the Frohberg yeast contained a high percentage of potassium, and a low one of calcium. The ratio is: \( K:Ca = 28:1 \) or, at least, 10:1. The yeast isolated from animal tissue had a relatively low percentage of potassium and a high one of calcium. The ratio is \( K:Ca = 9:14 \) or 1:1.55. This may explain why adding potassium chloride counteracts the reaction between glucose and phosphates, why calcium favors it when Frohberg's yeast is used, and why the opposite holds true for yeasts isolated from and adapted to animal tissue. Further results with other electrolytes will be published later.

G27. Slow Lactose Fermenting Bacteria Pathogenic for Young Chicks.

KEITH H. LEWIS AND E. R. HITCHNER, Department of Bacteriology, University of Maine, Orono.

A study has been made of the characteristics of a microorganism, isolated from several lots of infected chicks, which exhibited symptoms simulating pullorum disease. The delayed fermentation of lactose served to differentiate it from \( S. \) pullora and the other recognized fowl pathogens. The organisms are pathogenic for guinea pigs and young chicks and in the latter produce symptoms and pathological changes which resemble those in pullorum disease.

The cultural and biochemical tests were made according to the methods advocated in the Manual of Methods for Pure Culture Study. These bacteria are motile, Gram negative, non-capsulated, non-spore forming, facultative, short rods; grow well on ordinary nutrient media with characteristics closely resembling those of \( E. \) coli; curdle milk only after 20 to 30 days; reduce nitrates; form hydrogen-sulphide from peptone; do not liquefy gelatin; produce no indol; grow well in Koser's citrate medium; do not form acetyl-methyl-carbinol; methyl red positive.

Acid and gas are produced in 24 hours from arabinose, xylose, dextrose, levulose, galactose, maltose, trehalose, mannotose and sorbitol. Neither acid nor gas is formed from sucrose, cellobiose, raffinose, salicin, glycogen, dulcitol, adonitol and inositol. The appearance of acid and gas in lactose broth is delayed for 2 to 5 days. By aging the organisms for two weeks in lactose broth they acquire the ability to ferment this sugar in 24 hours. The aged organisms curdle milk more rapidly but in all other respects are identical with the original strains.
On Endo agar, purple lactose agar and Russell's double sugar agar the bacteria show characters which resemble those of the paratyphoid group. The organisms exhibit a remarkable tolerance toward Brilliant Green in that they grow readily on veal infusion agar in the presence of 1:25000 concentration of the dye.

Agglutination tests show little antigenic relationship of these organisms to E. coli, A. aerogenes, S. pullora, S. anatum, S. aertryke, S. enteritidis, S. schottmuelleri or Proteus vulgaris. Slow lactose fermenting bacteria, obtained from other workers, which show an apparent close antigenic relationship, have been distinguished from the avian strains by agglutination-absorption.

The bacteria exhibit characteristics which fail to fit well into any of the established genera of the Bacteriaceae and certainly are not identical with any of the recognized species. Because of the peculiar combination of characteristics displayed by this organism and the unsatisfactory state of bacterial nomenclature with respect to the slow lactose fermenting organisms no specific name is suggested.


Fuchsin lactose, formate ricinoleate, crystal violet, brilliant green lactose bile and Difco lactose broths were each tested separately, in parallel with standard lactose broth, pH 6.8-7.0, as routinely prepared in the Chicago Board of Health Laboratories. Two methods of testing were used: (1) Conventional inoculation from 61 coli-aerogenes stock strains, and (2) the Butterfield method, with results expressed by the Hoskins Most Probable Number, using a single stock strain of Bact. coli as the test organism.

Uniformly all trial and standard media produced gas within 42 hours of incubation at 37°C after the conventional inoculation of the 61 stock strains, in mass amounts. On this basis alone all the trial media would be approved for further study of their practical usefulness. The results by the Butterfield-Hoskins method, on the other hand, were quite different. This method is a quantitative one using a limited number of organisms for testing, and hence is more sensitive for the evaluation of media.

In order to compare the productivity of each trial medium with the standard medium impartially, proper allowance had to be made for a
normal variability in readings in any medium. To this end, the normal variation of standard lactose broth readings was determined. The mean percentage, minus deviation, 90.6, was arbitrarily taken as a minimum lower limit of variability to be met by more than half of the results with any given trial medium, in three or more successive tests. The most probable number for the trial medium divided by the corresponding value for the standard medium, gave the percentage value for comparison of each with the standard. The following trial medium values were obtained: fuchsin lactose broth: 33.8, 30.4, 14.5; formate ricinoleate broth: 95.9, 98.6, 90.1; crystal violet broth: 103.3, 113.5, 80.7, 71.6, 47.5; brilliant green lactose bile: 89.7, 108.7, 66.9, 82.3; Difco lactose broth: 114.4, 108.1, 100.5. Of these media only formate ricinoleate broth and Difco lactose broth met requirements for further study in these tests.

Each comparison required 50 tubes of the trial medium and 50 tubes of standard medium in each of three dilutions, or 300 tubes in all. The above data, therefore, represent observations on 5400 fermentation tubes. Similarly, 3600 tubes of standard medium were used in determining the range of normal variation of the readings.

G29. Effect of Temperature and Boric Acid on Gas Production in the Colon Group. Reese Vaughn and Max Levine, Department of Bacteriology, Iowa State College, Ames.

A medium containing boric acid is presented which may be useful for the detection of the genus Escherichia and to inhibit growth of the genera Aerobacter and Citrobacter. The medium has the following composition: Proteose peptone (Difco), 10 grams; lactose, 5 grams; K₂HPO₄ (anhydrous), 12.203 grams; KH₂PO₄, 4.085 grams; boric acid, 3.25 grams; Andrade's indicator, 10 cc.; and distilled water, 1000 cc.

The medium was sterilized at 15 pounds pressure for 15 minutes. The inoculum consisted of a 2.5 mm. loop of 24-hour nutrient broth culture of the test organism.

Of 148 strains of Escherichia studied 98 per cent grew luxuriantly in this medium after 48 hours at 43° to 44°C., whereas of 181 strains of the genus Aerobacter observed, only 3.3 per cent showed growth. Of 53 strains of Citrobacter studied there was no evidence of growth for any of the organisms.

There was some evidence of inhibition of gas production with some strains where growth did occur. Of the Escherichia cultures studied 95.3 per cent produced gas whereas not a single strain of the 181 Aero-
bacter or 53 Citrobacter cultures produced gas after 48 hours at 43–44°C. (temperature of the medium).


In work undertaken by the Sanitary District of Chicago a medium for isolation and quantitative estimation of E. typhi in sludge and sewage was needed. It was found that the older isolation media (Endo, E.M.B. and brilliant green agar) were too inhibitive of E. typhi when compared with nutrient agar and not sufficiently inhibitive of other sewage organisms. Wilson and Blairs' medium was found much too inhibitive of E. typhi, it being only 1.7 per cent as productive as nutrient agar. After many experiments with numerous modifications of the Wilson and Blair medium a satisfactory medium was found. This medium contains a lower concentration of the sugar, buffer, and all the inhibitive salts of the latest Wilson and Blair medium and contains a small amount of ferric chloride in addition. The iron salt is necessary to produce the characteristic E. typhi colony in the medium of lower concentration. This medium is 92 per cent productive of E. typhi and only 1.8 per cent productive of other sludge bacteria when compared to nutrient agar.

The medium base agar is prepared as follows: to 1000 cc. distilled water add peptone, 5 grams; beef extract, 3 grams; glucose, 3 grams; Na₂HPO₄·12H₂O, 2 grams; washed agar, 20 grams. Boil until solution is complete; bottle in 150 cc. amounts (without clarification) and sterilize at 15 lbs. for 15 minutes. pH adjustment is unnecessary.

The following stock solutions are prepared to be used with the medium:

(1) Bismuth citrate, 3 per cent solution. This is made by boiling 30 grams of bismuth citrate (U. S. P.-Merck) in 1000 cc. of water and adding aqua ammonia (sp. gr. 0.90) until solution is complete. About 30 cc. of ammonia is required. (2) A 20 per cent Na₂SO₃ solution. (3) A 1.0 per cent ferric chloride solution, 2 per cent if FeCl₃·6H₂O is used. (4) A 1.0 per cent brilliant green solution.

For convenience the above solutions are added from two "mixes" as follows: Mix A consists of 36 cc. of solution (1) and 38 cc. of solution (2) and 26 cc. of sterile distilled water. Mix B is prepared by adding 12 cc. of solution (4) to 100 cc. of solution (3).

When the base agar is melted, mix A is shaken and 10 cc. is added per
150 cc. of agar. This is followed by 3 cc. of mix B. The medium should then be cooled to 45°C and added in 10 cc. amounts with a pipette to the plates containing the samples under examination, care being taken to keep all the precipitate in suspension during the distribution of the medium.

Plates poured with the medium are incubated for 20 to 24 hours at 37°C. *E. typhi* colonies are small with a typical delicate brown halo. Certain other sulphite reducers produce larger colonies with a slightly darker brown halo. Suspicious colonies are counted and fished to Russell's double sugar medium slants and are further confirmed by other fermentation tests and agglutination.

*E. typhi* has been isolated from Chicago activated sludge with this medium. Death rate curves of *E. typhi* in stored activated sludge have been obtained. *E. typhi* has been isolated from sludge samples with this medium when for each *E. typhi* present there were 1000 other members of the coli-aerogenes group and 25,000 other bacteria.


The use of the sodium thiosulphate treated sample bottle in collecting samples of water from chlorinated swimming pools has demonstrated that during periods of heavy bathing loads, large numbers of bacteria are present, as revealed by 37°C. total counts, *Escherichia coli* indices and streptococci indices. Data are presented to show the effect of varying bathing loads at different chlorine residuals in different sized pools. By regulating the bathing load, the bacterial contents of the pools could be held within the present standards recommended by the American Public Health Association Committee on Bathing Places. A minimum of 0.2 p.p.m. available chlorine was found to be low. In general, the chlorine residual should be maintained at 0.4 p.p.m. The critical period of pollution was found to occur shortly after the bathers entered the water.


A new medium, consisting of: 0.5 per cent peptone, 0.5 per cent lactose, 0.5 per cent sodium formate and 0.1 per cent sodium ricinoleate, has been used for the detection of colon organisms in water and milk.
This medium inhibited the growth of all of those organisms, tested by us, which have been mentioned by other workers as being responsible for "false tests." The medium accelerated the growth of the bacteria belonging to the Escherichia-Aerobacter group, caused the total population of bacteria to be much larger, and very markedly increased the total amount of gas produced.

The sodium ricinoleate inhibited the growth of the bacteria which may cause positive presumptive tests which can not be confirmed. Yet the results of our experiments indicated that when one colon organism is present growth will occur and large amounts of gas will be produced.

Sodium formate accelerated growth and caused the total number of bacteria to be larger, partly because of its buffering effect. The pH of the medium after two days' incubation was above 6.0. The amount of gas produced was increased because bacteria belonging to the Escherichia-Aerobacter and Salmonella groups can produce gas from formic acid, in the absence of any other fermentable substance. Gas production from the formate resulted in the accumulation of NaOH and NaHCO₃, which substances maintained the high pH in the medium.

The addition of the amounts of protein material which would of necessity be added to the medium in testing water or milk for the presence of colon organisms, did not materially change the surface tension of the medium.

Formate ricinoleate broth has been used very satisfactorily in testing several hundred samples of water and milk.


Standard lactose broth has been criticized because it permits the growth of gas-producing organisms of no known sanitary significance. Media, containing different dyes in various amounts, have been suggested to replace lactose broth.

Salle's crystal violet broth, Dominick and Lauter's broth, gentian violet bile broth and brilliant green bile (2 per cent) broth have been studied by us to determine: (1) the growth inhibiting and growth accelerating substances in these complete media and their various constituents; (2) the ability of small numbers of colon organisms to initiate growth in the media; and (3) the detoxifying action caused by adding to tubes of the broths the amounts of milk (0.1 and 1.0 cc.) which would be added to the media when they are used in the detection of colon organisms in milk.
Crystal violet, methylene blue, gentian violet and brilliant green were shown to be the growth inhibiting substances in the media. Bile was found to decrease the toxic action of the dyes used and to accelerate the growth of "false test organisms" in media in which oxgall was used.

Small numbers of colon organisms were able to initiate growth and produce gas in brilliant green bile broth. Small numbers of these organisms did not grow and produce gas in Salle's crystal violet broth.

Many bacteria responsible for "false tests" were able to grow in these media. The addition of 1 cc. of sterile milk to tubes of the media materially increased the number of "false test organisms" which were able to grow. Failure to inhibit the growth of these gas-producing bacteria, not known to be of any public health significance, is a major weakness in the media.

G34. Eosin Methylene Blue Smear Agar for Rapid Direct Count of E. coli.
H. W. Gehm and H. Heukelekian, New Jersey Agricultural Experiment Station, New Brunswick.

The standard eosine methylene blue agar for the confirmation of E. coli has been used for the direct count.

The obvious difficulties involved in using this medium for direct count by the pour method have led us to employ a smear method which gives more characteristic surface growths. The various dilutions are smeared on the hardened surface of this medium, allowed to dry and incubated for 24 hours.

After 24 hours incubation characteristic colonies are counted. Good distribution was observed in all cases, and very few other organisms developed. The counts obtained in 24 hours were consistently higher than those by the 48-hour brilliant green tube method but proportional to them. Incubation for an additional 24 hours gave few additional E. coli colonies on the plates.

MEDICAL BACTERIOLOGY, IMMUNOLOGY AND COMPARATIVE PATHOLOGY

M1. The Place of Bacterial Allergy in the Immunization Process. L. Dienes, Massachusetts General Hospital, Boston.

The immunization process does not start with the production of antibodies and their diffusion in the tissues and body fluids, but with a period of pure tissue hypersensitivity the development of which precedes the production of circulating antibodies a few to many days. Guinea pigs after injection of a few milligrams of egg white give a slight