The writer thought it might be of interest to prepare the analog of phenolphthalein by condensing quinolinic anhydride with phenol, and to ascertain whether the resulting product would have the properties of an indicator.

Quinolinic acid was converted into its anhydride by heating with acetic anhydride and washing out the acetic acid and excess of acetic anhydride by means of carbon tetrachloride. Phillips[1] recommends heating one part of the acid with two parts of acetic anhydride to 120°, then gradually raising the temperature to 150°. The writer found that a quantitative yield of the anhydride was obtained by gently heating the mixture until solution was effected, then simply boiling for five minutes. On cooling, the anhydride separated out, and after washing with carbon tetrachloride, it showed the correct melting point of 134°.

Ten grams of quinolinic anhydride, 20 g. phenol, and 8 g. of concentrated sulfuric acid were heated in an oil bath at 120° for ten hours. The mixture was then poured into water, and the solution boiled until the excess of phenol was expelled. A yellow granular precipitate formed, which was collected on a filter, then dissolved in alcohol and purified by boiling with charcoal. A nearly colorless solution was obtained, which on evaporating and diluting with water became milky and finally yielded a yellowish granular sediment. The product was analyzed for nitrogen by the Kjeldahl-Gunning method.

Calc. for $C_{18}H_{13}NO_4$: N, 4.56; found, 4.50.

Like phenolphthalein, phenolquinolinein is a brilliant indicator, giving an intense pink color with alkalies which is immediately discharged on acidifying. On account of the basic nature of the pyridine nucleus, the end point might be expected to be somewhat different from that of phenolphthalein, but this was not determined. On account of the present cost of quinolinic acid, it is not probable that the indicator will find any extensive application in titrimetric work. The analogy, however, is considered of sufficient interest to warrant this brief paper.

IOWA STATE COLLEGE,
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY.]

# STUDIES ON THE CULTURE MEDIA EMPLOYED FOR THE BACTERIOLOGICAL EXAMINATION OF WATER.
## II. LACTOSE-PEPTONE MEDIA.[2]

BY E. M. CHAMOT AND C. M. SHERWOOD.

Received June 17, 1915.

The most important of all the qualitative methods for the bacteriological examination of water are unquestionably those in which advantage is

[1] *Ann.*, **288**, 255 (1895).

[2] Read at the Rochester Meeting, American Chemical Society, September, 1913.

taken of the fermentative action of the microörganisms upon carbohydrates. In this class or group of media those in which lactose is used in combination with some nitrogenous substance are more often employed than any other. The diagnostic features upon which marked emphasis has been laid are: The total volume of gas produced; the rate of gas formation; and the composition of the gas, especially the volume proportion of $CO_2$ to gases not absorbed by alkalies; this latter proportionality has been termed the gas ratio. The total volumes of gas formed and the gas ratios obtained by different investigators, when working with the same bacterial species as described in the literature, agree fairly well, yet the variations are of sufficient magnitude to be beyond the limits of personal error. A careful search through the literature failed to show any systematic studies of the effects of the concentration of the different components upon the gases formed or of their effects upon the gas ratios. It appeared, therefore, that some interesting facts might follow the application of the methods described in the first paper of this series[1]—that is to say, a study, by means of the triangular diagram as a guide, of the concentration of nitrogenous material, of inorganic salts, of acidity, and of carbohydrates in the media. It is obvious that such media are in reality four-component instead of three-component systems; but for the purposes of the investigation it was thought possible to hold one component at a time constant, and vary the other three. The method employed has been described at length and in detail in the first paper and need not here be discussed.

Since the most important of the fermentative bacteria, from the viewpoint of the water analyst, are those of the *B. coli* group derived from the feces of man and animals, our studies were confined to this group. In the greater part of the work we employed an artificial "sewage" thus prepared: One loopful of fresh moist feces (approximately 0.02 g.) was thoroughly shaken with 100 cc. of sterile water, filtered through sterile muslin into a sterile one-liter measuring flask and diluted to the mark with water from the University water supply pipes. This polluted water gave at different times from 2000 to 5000 colonies on standard gelatin at 20° at the end of forty-eight hours' incubation; approximately 200 colonies on agar at 38°, of which from 20 to 40 colonies proved to be assignable to the *B. coli* group.

In addition to this source of fecal bacteria, various polluted waters were tried, normal sewage, waters polluted with the feces of various domestic animals; and in addition fermentation tubes were inoculated with strains of pure cultures isolated from various sources.

Preliminary to the main investigation it was essential to determine whether under the conditions which would obtain in the preparation and

[1] THIS JOURNAL, 37, 1606 (1915).

sterilization of the culture media any marked inversion of the lactose would occur. With this end in view a large number of determinations were made upon culture media before and after sterilization, both in streaming steam in an Arnold sterilizer and also under pressure in an Adnet autoclave at 110° and 120° during periods of from 15 to 30 minutes. No change in the lactose could be detected with the low acidities employed, either by a sensitive Schmidt and Haensch polarimeter, or by gravimetric determinations of lactose by the Allihn or by the Deferen-O'Sullivan methods.

When the media were prepared from lactose, neutral inorganic salt and Witte peptone alone, with the reaction unadjusted (*i. e.*, having a final acidity of from 1 to 1.2% due to the peptone), no perceptible change in acidity was detected after sterilization, care of course being taken to make all titrations at the same temperatures,[1] but with meat and liver broths marked changes in acidity always resulted during sterilization and upon subsequent standing.

In the course of the investigation, distilled water, water obtained by redistilling the laboratory-distilled water from acid permanganate, and water from the taps of the University water supply were in turn employed. Since the last source gave us somewhat more sensitive media it was employed in practically all the work hereinafter described.

The first series of experimental runs, using the triangular diagram as a guide, were undertaken with peptone, lactose and potassium chloride or sodium chloride as variables, the acidity being maintained constant in all the series at a value equivalent to 1% *N* HCl. The most rapid and most uniform fermentation was obtained in those media containing from 3 to 3.5% peptone, 0.6 to 1% lactose and 0.5 to 1.5% KCl or 0.5 to 1% NaCl, KCl being decidedly preferable.

**Effect of Variations in Initial Acidity.**—It having been ascertained, as stated below, that the lactose should be present in a medium in a concentration lying between 0.6% and 1%, it became possible by adopting a mean of 0.8% lactose in all media, to study, by means of the triangular diagram, the effect of the initial acidity. Runs, therefore, were made with concentrations plotted clock-wise upon the diagram as follows: Peptone, 1 to 6%; acidity, 0 to 2.5%; potassium chloride, 0 to 2.5%; lactose, 0.8%. In all the experiments tried, numbering several hundred, the most rapid gas formation and hence the first appearance of gas took place in media having an initial acidity of between 0.5% and 1.5%, expressed in cubic centimeters of *N* HCl with phenolphthalein as indicator. Other diagrams were now studied with peptone concentrations

[1] With the media as prepared in our laboratory a 3% peptone solution has a reaction of approximately +1% at 20°, +1.1% at 37°, and +1.5% at boiling (approximately 98°).

varying from 2 to 4%; potassium chloride, 0 to 1%; acidities, 0 to 10%. As before, gas appeared first and reached its maximum most rapidly in acid media. Neutral media yielded the greatest gas volumes,[1] but the gas was slower in appearing and showed greater variations in the final gas volumes than was the case of media having an acid reaction. An acidity equivalent to 1.5% appeared to be the upper limit of usefulness of media for diagnostic purposes. With low acidities media containing 3, 3.5 and 4% peptone yielded gas volumes equivalent to over 50% of the closed arm of the fermentation tubes. The effect of initial acidity upon the total gas volume of a 3% peptone medium is shown graphically in Fig. 1, in which the average results obtained from a very large number of runs have been plotted. Proportionally similar results were obtained with higher peptone concentrations. In all the experiments made the addition of potassium chloride appeared to be distinctly beneficial.

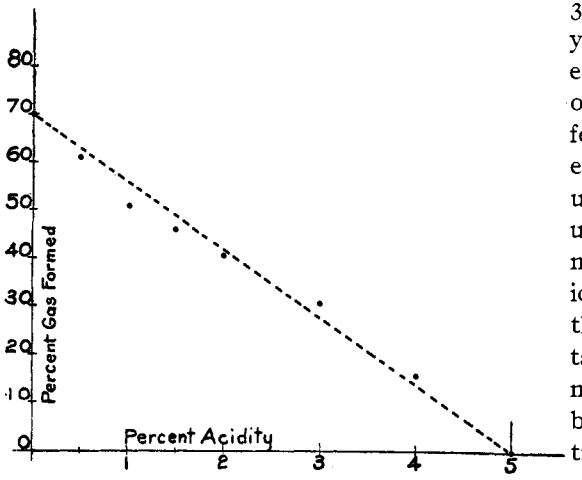

Fig. 1.

**Effect of Lactose Concentrations.**—Since the reading of this paper W. W. Browne[2] has published the results of his investigation upon acid formation which has led him to reach the conclusion that 1% carbohydrate in a medium is sufficient for the maximum production of acid by members of the *B. coli* group. This is in agreement with the results obtained by us on gas formation. Stamm[3] has found that from 0.5 to 3% of dextrose made little appreciable difference either in the total gas formed or in the gas ratio.

We tried systematically various media containing from 0.1% to 10% lactose. The results obtained confirmed those of Stamm with dextrose. Above 0.4% lactose up to 3% no appreciable effects upon gas volumes or upon rapidity of fermentation could be noted. Under 0.4% the gas volumes obtained were not uniform and the total gas formed occupied a

[1] W. W. Browne has found similar results as to total acidity produced. Neutral and slightly alkaline media yield a greater final acidity than media, having an initial acid reaction. See "Acid Production by *B. coli* Group," *J. Infect. Dis.*, **15**, 580 (1914).

[2] Browne, *Ibid., Loc. cit.*

[3] Stamm, *Centr. Bakt. Parasitenk*, **42**, 590 (1906).

less volume of the closed arm than when lactose was present in higher concentrations. When 3% was reached a retardation in the rate of gas formation was noted and above this value the inhibiting effect was quite marked. For diagnostic purposes in water and sewage examinations the optimum conditions as to rapidity, large total volume of gas, and uniformity of results, appeared to lie between 0.6% and 1% lactose.

It was thought desirable to ascertain approximately the quantity of lactose fermented by the mixed flora of sewage in the diagnostic tubes. With this end in view, portions of lactose-peptone media were precipitated by phosphotungstic acid, filtered and the carbohydrate present in the filtrate determined by both the Deferen-O'Sullivan gravimetric method and by means of the polarimeter. Other portions of the same media were placed in fermentation tubes, inoculated with sewage, incubated at 37 to 38°, and as soon as no further gas increase could be detected the unchanged carbohydrate was determined in the media in the same manner as before. In the case of the inoculated and incubated samples the polarimeter gave slightly lower values for the lactose fermented than did the gravimetric method, doubtless due to the formation of optically active compounds formed in the fermentation of the lactose or in the decomposition of the peptone. It was found that from 25 to 35%, of the lactose in 1% lactose media was all that the bacteria had converted into gas and other products. In other words, lactose media in practical work must contain approximately 0.6% lactose while any greater concentration than 1% is useless and uneconomical.

**Effect of the Presence of Inorganic Salts.**—The influence of the presence of NaCl and KCl in carbohydrate media in stimulating a more rapid fermentation by sewage organisms and assuring a greater uniformity in the total gas volumes formed has already been alluded to above. The methods employed in the study of the influence of the different inorganic salts available have also been described in our first paper.

As a result of our experiments upon lactose media with a mixed sewage flora and with pure cultures of members of the *B. coli* group, it is possible to arrange the inorganic salts tried into two groups:

I. Salts hastening fermentation and usually conferring greater uniformity of gas production.

| | |
|---|---|
| KCl | in concentrations up to 3.0% |
| $K_2SO_4$ | in concentrations up to 3.0% |
| NaCl | in concentrations up to 1.5% |
| $Na_2SO_4$ | in concentrations up to 2.0% |
| $MgSO_4$ | in concentrations up to 2.0% |
| $CaCl_2$ | in concentrations up to 1 % |

II. Salts having little influence upon gas production or acting as inhibiting agents.

$KNO_3$, $NaNO_3$, $NH_4NO_3$, $NH_4Cl$, $(NH_4)_2SO_4$; $MgCl_2$; $MnCl_2$; $MnSO_4$.

In Group I when the maximum concentration stated is approached an inhibiting effect is noticeable and if concentrations above these values are employed both the rate of fermentation and the final gas volume are reduced.

Potassium chloride having yielded the most satisfactory results, special runs were made with lactose at 0.8%; acidity, 1%; peptone, 3, 3.5 and 4% and with the KCl, 0 to 4%; each concentration differing from the next by 0.25%. Checks were also made, using NaCl. The results may be summarized as follows: (1) The addition of KCl lessened the time required for the first appearance of gas by from three to ten hours. (2) The total gas formed upon completion of fermentation was much more uniform in the different fermentation tubes containing media of like concentrations when KCl was present than where absent. (3) KCl appeared to be preferable to NaCl for rapid diagnostic purposes. (4) The best results were obtained with media containing from 0.5 to 1.5% KCl. (5) The total gas formed varied with the per cent. of peptone present but a 3%

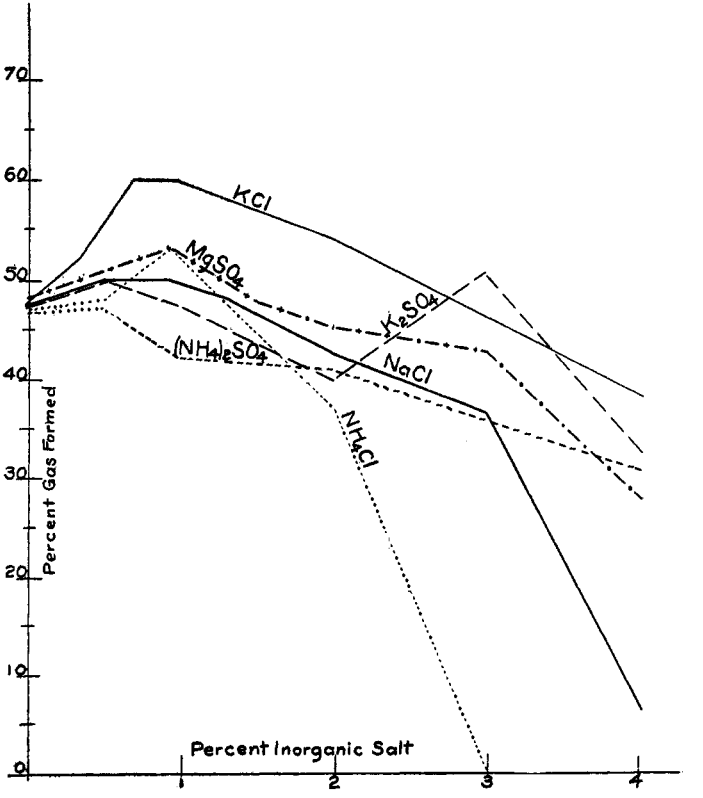


Fig. 2.

peptone from the viewpoint of rapidity and accuracy of diagnosis appeared to be as satisfactory as higher concentrations.

The average results of several hundred series of experiments using 3% peptone media and six typical inorganic salts are shown graphically in Fig. 2. It will be seen that in all cases a slight addition of an inorganic salt appears to stimulate fermentation.

The addition of salts of phosphoric acid to lactose-peptone media gave either no appreciable effects in very low concentrations or decidedly inhibiting effects in high concentrations.

The following phosphates were tested in different concentrations: $Na_2HPO_4$, $NaH_2PO_4$, $NaKHPO_4$, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, $K_2HPO_4$, $KH_2PO_4$, $MgHPO_4$, $NH_4MgPO_4$, $CaHPO_4$. The concentrations of these phosphates which could be added without causing very voluminous precipitates during sterilization were found to be very low; usually as little as 0.02% produced a muddy unsatisfactory medium. In no case could we introduce a phosphate in concentrations above 1%. The salts $KH_2PO_4$, $K_2HPO_4$, $MgHPO_4$ and $NH_4MgPO_4$ appeared to exert a slightly stimulating action upon gas production but in all the experiments tried the final gas volumes lacked uniformity save in the case of $NH_4MgPO_4$ alone. The last-named salt gave us better results than any other phosphate tried, but not so good as with media containing NaCl or KCl.

It may, therefore, be safely concluded that the amount of $PO_4$ ions present in peptone-containing media as ordinarily prepared is sufficient to meet the needs of a mixed sewage flora. Even so-called C. P. lactose usually contains phosphates; that employed in this investigation was found to contain 0.01% $PO_4$. When a natural water instead of distilled water is employed a still further addition of $PO_4$ almost invariably results.

**Effect of Peptone Concentrations upon Gas Volumes.**—When the concentrations of inorganic salts do not exceed 1% and the acidity is maintained at between 1% and 1.5%, the total gas volume formed is proportional to the concentration of the peptone, meat, liver, or beef extract added until a point is reached where the viscosity is so great, or the concentration of certain materials present in these substances is so high, as to seriously interfere with the development of the mixed flora found in sewage.

In the results averaged in the curves shown in Fig. 3,[1] from some 3000 experimental inoculations, the lactose was kept constant at 0.8%, the acidity at 1%, the inorganic salt, in this case KCl, at 0.6%. The peptone was varied between 1 and 10%. Under these conditions there is a very

[1] In Fig. 3 the gas volumes indicated by the black dots are averages of results obtained with fermentation tubes of about 300 cc. closed arm capacity, used in the study of the composition of the gases of fermentation. The small circles are the averages of gas volumes obtained with ordinary fermentation tubes.

rapid rise in the total gas formed until a concentration of about 4% is reached, after which the increase in volume is slow and becomes approximately constant at a little over 10% peptone. Above 5%, however, the rate of fermentation is materially reduced and with these high concentrations the fermentative process, in some cases, may not be completed for several days. The most rapid evolution of gas and completion of the fermentative process was always observed with concentrations of between 3 and 4% peptone.

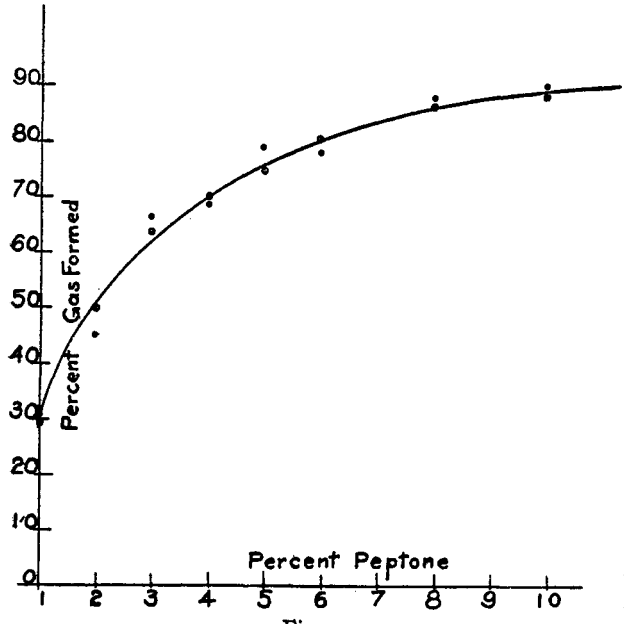

Fig. 3.

In the opinion of the authors these results are the most interesting and important of the entire investigation. For it is obvious that a very slight variation in the proportion of peptone in low concentrations will result in a relatively great variation in gas volumes. Since peptone is very hygroscopic and since in the preparation of culture media it is the usual practice to exercise little care in weighing out this component, it follows that the media made at different times are apt to vary considerably in the nitrogenous component. A marked variation in the volume of gas formed will therefore result.

Water analysts have always believed that if great care is exercised in adjusting the acidity, this is sufficient to standardize the media. It appears, however, that for diagnostic purposes it is essential to use greater care in the proportioning of other components and that in reality a slight error in acidity between the limits commonly employed will effect the results much less than a variation in the concentration of the nitrogenous components, a fact which appears to have escaped attention.

Nor does this phenomenon apply to peptone alone for we have obtained similar increases in gas volumes with an increase in the proportion of beef flesh or beef liver. The results of these runs are shown in Fig. 4. In media prepared with beef or with beef or calves' liver, lactose was added in the

proportion of 0.8%, the reaction adjusted to +1% and in most of the runs 0.6% of KCl was added. When both peptone and meat or liver were used the change in gas volume in proportion to the concentrations was even more marked than in unpeptonized media. Since it may be said to be practically impossible to purchase at a butcher shop meat twice alike as to composition, that is, as to proportion of flesh, fat, connective tissue, moisture, etc., it follows that we must expect at different times a variation of at least 10% in the final gas volumes unless we employ higher concentrations than is the rule. Moreover, we will show in our next paper that the composition of the gases of fermentation vary with the concentration of the nitrogenous components and therefore that the "gas ratio" obtained will depend upon the character of the medium employed.

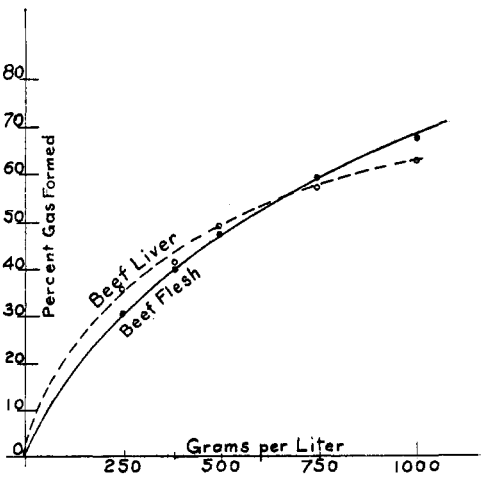

Fig. 4.

Media made with commercial "extract of beef" alone also yield larger gas volumes in higher concentrations. But in our hands the results have been exceedingly variable, and the total gas volumes usually small and, therefore, of no diagnostic values. Media containing high concentrations ferment very slowly; this is not surprising since their phosphate content is excessively high, so high in fact that large amounts of $NH_4MgPO_4$ invariably separate during the adjustment of the reaction and subsequently in the fermentation tubes; furthermore, the acidity of this material is so high that upon neutralization to a reaction equivalent to +1% of *N* HCl a large addition of inorganic salt is made to that already present. Media made from commercial extract of beef, therefore, are apt to contain too high a concentration of inorganic salts other than phosphates and too low a concentration of the sort of nitrogenous matter necessary for the growth of fermentative bacteria. The fermentative process is therefore partially inhibited and the gas formed variable in volume and in composition.

**Influence of the Size of Fermentation Tubes upon Gas Volumes.**—The total volume of gas which collects in the closed arm of the fermentation tube is by custom recorded and spoken of in terms of the length of this closed arm occupied by the gas. For convenience it is commonly expressed in per cent. This volume per cent. has been regarded as constant,

within narrow limits, for a given bacterial species and therefore is believed to be of considerable diagnostic value. We have pointed out above that the final gas volume is proportional to the per cent. of available nitrogenous material present in the medium. It remained to ascertain whether this held good for different styles of fermentation tubes. Excessively long (25 cm.) and excessively short (5 cm.) tubes were employed; tubes with diameters ranging from 4 mm. to 60 mm.; tubes containing 5 cc. up to 500 cc.; but in all cases we found that the gas volumes expressed in per cent. of the closed arms were substantially constant for a given peptone or meat concentration and that this per cent. appeared to be independent of the size of the closed arm of the tube, when the tube is of uniform diameter.

**Composition of the Gases Formed.**—Since the volume of the gas formed increases up to a certain maximum with an increase in the concentration of the peptone, meat, liver or meat extract present, and since the ratio of gases absorbed by KOH to those unabsorbed is employed in diagnosis, it became imperative to make careful analyses of the gases formed. The results obtained will be discussed in the third paper of this series. It will there be shown that the per cent. of $CO_2$ in the gas produced by sewage bacteria increases and the hydrogen decreases with the concentration of the nitrogenous material until a certain concentration is reached when the $CO_2$ and H no longer change.

## Summary.

1. In the fermentation of lactose by bacteria in water contaminated by sewage, human feces, the feces of domestic animals, and pure strains of the *B. coli* group, the total volume of gas formed increases to a final maximum with the concentration of the peptone, meat, liver, or meat extract employed.

2. The composition of the gas formed is dependent upon the concentration of the nitrogen-containing substance employed.

3. The addition of from 0.5 to 1% of KCl to lactose-peptone media appears to stimulate fermentation and assure more uniform results.

4. Similar beneficial effects are obtainable with NaCl, but of not so marked a character.

5. Nothing is to be gained by employing a lactose concentration of over 1%.

6. Neutral media appear to yield slightly greater gas volumes than media slightly acid to phenolphthalein; but media having a reaction of approximately +1% ferment considerably more rapidly and yield diagnostic results in several hours' shorter time.

7. The gas ratios of organisms of the *B. coli* group are dependent upon the concentration of the peptone or other similar nitrogenous material in the media.

8. The addition of meat infusion to peptone media improves this media

when low concentrations of peptone are employed, but yields media whose reactions rapidly change.

9. A very sensitive peptone culture medium yielding uniform results and large gas volumes consists of peptone 3 to 4%, lactose 0.8%, potassium chloride 0.6%, reaction +1%. Such media show little change on keeping.

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[CONTRIBUTION FROM THE ROBERT HARE LABORATORY OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA.]

# THE DISTRIBUTION OF ARSENIC IN LIVER TISSUE IN CASES OF POISONING.

BY LEON A. RYAN.

Received June 14, 1915.

This investigation was undertaken to ascertain if arsenic administered to an animal, either in its food or by subcutaneous injections, is equally distributed in the liver tissue of the animal. From the nature and structure of the liver it would most naturally be expected that the arsenic would be uniformly distributed throughout this organ.

**Dog No. I.**—A male dog, weighing 18 pounds, in which a permanent biliary fistula had been established and the common duct ligated with another object in view, received subcutaneous injections in the neck, shoulders, abdomen and legs of various volumes of a solution of sodium arsenite equivalent to 0.001 mg. of arsenious oxide per cubic centimeter. The total amount of sodium arsenite solution injected in different parts of the body during seven successive days was equivalent to 191.5 mg. of arsenious oxide. Death of the dog occurred on the eighth day.

Immediately after the death of the dog an autopsy was made. The liver appeared of normal size for a dog weighing 18 lbs. No abnormal coloring of the liver substance was observed. The weight of the liver immediately after removal was 338.7 g.

The liver tissue in the case of each dog was decomposed by the Fresenius-von Babo method.

DOG NO. 1.

| Part of liver analyzed. | Moist wt. in grams. | Gram $As_2S_3$ obtained. | Equiv. to $As_2O_3$. | Per cent. $As_2O_3$ in moist tissue. |
|---|---|---|---|---|
| Left lateral lobe | 80.6 | 0.0018 | 0.0014 | 0.0017 |
| Left central lobe<br>Right central lobe | 115.78 | 0.0023 | 0.0018 | 0.0015 |
| Caudate lobe<br>Right lateral lobe | 121.39 | 0.0027 | 0.00217 | 0.00178 |

**Dog No. II.**—A solution of sodium arsenite containing progressively increasing quantities of the compound was mixed with cracker crumbs and, with other food, fed to a dog weighing 24 pounds. The administra-