in securing a salt of constant composition, and, as we know from the work of Lemoult and others, conditions must be very rigorously maintained in order to secure any given salt of cyanuric acid in a state of purity.

For these reasons we believe that the tetracarbonimide of Scholtz is only cyanuric acid.

The authors take pleasure in acknowledging their indebtedness to Professor C. H. Warren, of this Institute, for invaluable assistance in that portion of this investigation dealing with the optical characteristics of crystals.

## Summary.

1. It has been shown that uric acid can be oxidized by hydrogen peroxide in such a way as to yield cyanuric acid to the extent of about 50% of the theory.

2. The cyanuric acid thus obtained has been subjected to an exceptionally thorough identification.

3. The accounts of previous investigators who have described tetracarbonimide as a product of the reaction show no conclusive evidence that they were dealing with any other compound than cyanuric acid. It is therefore concluded that the compounds are identical.

4. A systematic study of the reaction of hydrogen peroxide upon uric acid and its derivatives is already far advanced in this laboratory and we desire to reserve this field.

CAMBRIDGE, MASS.

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[CONTRIBUTION FROM THE LABORATORY OF SANITARY CHEMISTRY, CORNELL UNIVERSITY.]

# STUDIES ON THE CULTURE MEDIA EMPLOYED IN THE BACTERIOLOGICAL EXAMINATION OF WATER.

## IV. NEUTRAL RED LACTOSE PEPTONE MEDIA.

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

Received June 22, 1917.

Neutral red was discovered by Witt[1] in 1879 and further investigated by Bernthsen and Sweitzer[2] in 1886, but not until 1898 was it applied to bacteriological diagnosis. In that year Rothberger[3] described the effects of various organisms on thirty-five dyes and showed that while some organisms were able to cause a change in color when inoculated into the media containing certain of these substances, others produced no change. As a result of this investigation he recommended bouillon agar containing neutral red as a medium for distinguishing between *B. coli* and *B. typhosus*. At 37° *B. coli* causes a ruby-red colored medium to change to a canary-

[1] *Ber.*, **12**, 931 (1879).

[2] *Ibid.*, **19**, 2604 (1886).

[3] *Centr. Bakt. Parasitenk, Abt. I*, **24**, 513 (1898); **25**, 15, 69 (1899).

yellow, while, at the same time, a strong fluorescence is noticeable, but *B. typhosus* does not change the original color nor does it bring about a fluorescence in the medium. When *B. coli* and *B. typhosus* are mixed together in various proportions the reaction appears identical to that produced by *B. coli* alone. Most of the other pathogenic organisms studied give negative results.

Since 1898 the medium of Rothberger has been studied and modified by a considerable number of investigators. Stokes,[1] however, while studying the use of neutral red for the identification of *B. coli* in water, made such important changes in the medium that a brief summary of his work is essential. While the previous investigators had used a neutral red medium either in the form of dextrose broth or agar shake cultures, the medium being placed in ordinary test tubes, Stokes was the first, we believe, to conceive the idea of placing in a Smith fermentation tube a sugar medium containing neutral red. This gave additional diagnostic characteristics in that one could use not only the reduction of neutral red but also the quantity of gas produced and the ratio of carbon dioxide to hydrogen in this gas. Stokes tried dextrose, lactose, and saccharose media. With the first two sugars the change in the neutral red was particularly marked and consisted in a conversion of the red color of the sugar bouillon in the closed arm of the fermentation tube into a clear canary-yellow, or at times a darker orange color. The open bulb remained red or assumed a port-wine color, and a sharp line of demarcation between the yellow and red colors took place in the stem uniting the closed and open bulbs. Stokes recognized the selective value of lactose for *B. coli* and recommended its use in diagnosis. When this sugar was used, one had the gas production, ratio of carbon dioxide to hydrogen, and the characteristic contrasting color change upon which to base a diagnosis. Stokes examined 567 gas formulas and found only six of these corresponding to the formula for *B. coli* in lactose bouillon which failed to give the other tests for the colon bacillus. Three of these turned neutral red entirely yellow in both closed arm and bulb of the fermentation tube, leaving only one of the 567 specimens (two not tested in lactose) which could have been mistaken for "colon" if direct inoculation of the fermentation tubes had been employed without further plating and isolation.

Rochaix and Dufourt[2] made a rather extended study of the applicability of neutral red to the detection of fecal pollution in water, and decided that the change in neutral red, if it is not always an indication of the colon group, does show pollution by urine and fecal matter. They were, however, apparently unaware of the result of Stokes' researches

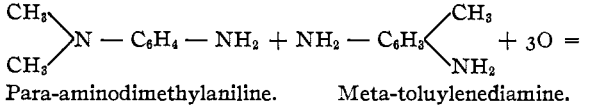
[1] *J. Infect. Dis.*, 1, 341 (1904).

[2] *Compt. rend. soc. biol.*, 69, 312 (1910).

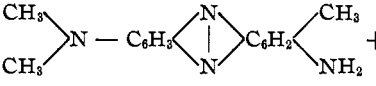
and continued the use of dextrose in the open tube thus making their results of much less value.

Although the applicability of neutral red to bacteriological diagnosis has been quite extensively investigated, very little attention has been paid to the chemical nature of the reaction when the dye changes from the distinct red color to a fluorescent yellow under the influence of certain bacteria. Rothberger, apparently, assumed the change to be caused by reduction, due to the organisms, but made no attempt to explain the reduction or to duplicate it by artificial means. Irons[1] also regarded the color change as due to simple reduction and concluded that, from the nature of the reaction, the method was not specific for *B. coli*. Gage and Phelps[2] were the first to attempt a study of the exact nature of the chemical reaction involved when neutral red is acted on by *B. coli*. These investigators state that the production of the yellow color has been ascribed by various authors to one of two causes: (1) The yellow fluorescent color is due to the alkali produced by the bacteria. (2) The color change is due to reduction. They point out that the first hypothesis is incorrect because the bacteria produce the color in 5% acid solution and the yellow color formed by an alkali is not fluorescent. They further state that the second hypothesis is erroneous, if reduction in the true chemical sense is meant, because the first reduction product possible is toluylene blue, an indamine body in which the two benzene rings are held together by only one N atom while a continued reduction causes a separation of the rings into the amino bodies from which the dye is made.

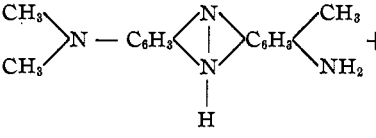
They give the following as the reaction involved:



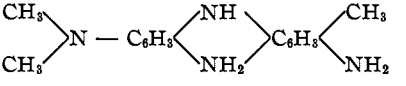
Para-aminodimethylaniline. Meta-toluylenediamine.



Neutral red. (1)



Toluylene blue. (2)



Yellow body. (3)[3]

The only one of the components which could be confused with the

[1] *J. Hyg.*, **2**, 314 (1902).

[2] Am. Pub. Health Assoc., *Rept.* **28**, 402 (1902).

[3] *Ibid.*, *Rept.* **28**, 410 (1902). These formulas, it is obvious, contain typographical errors.

yellow compound produced by the bacteria is (3), but the authors point out that it differs from the bacterial product in two important particulars: (1) The yellow body thus formed is not a dye, while the yellow substance produced by the bacteria is. (2) On exposure to air the chemically formed yellow body oxidizes to brown powder and not to the neutral red as the bacterial product does. They conclude that the yellow bacterial product will be found to belong to an entirely new series, possibly one of the fluorescene series.

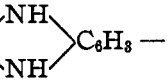
In 1911 Guerbet[1] published an article on the chemical nature of the *B. coli* neutral red reaction in which he states that it is simply one of reduction by the organisms, the fluorescence being due to the phenomena of reduction plus the cloudiness of the culture, and the yellow tint to the presence of ammonia in the culture; but he makes no statement as to the probable change in the structure of the neutral red molecule during the reduction process.

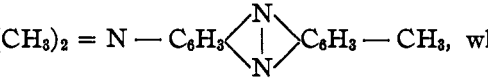
It is evident from this brief outline that no really satisfactory conclusion had been reached as to the chemical change taking place which causes the fluorescent yellow compound to be formed.

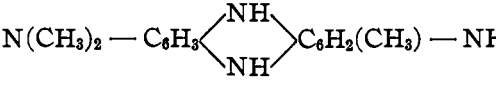
Neutral red—dimethyldiaminotoluphenazine—belongs to a series of compounds known as the eurhodines. They are the simplest of the azine dyes and consist of two rings joined to the azine group with the N symmetrically attached to the rings  . The base of these compounds is phenazine, $C_6H_4$  $C_6H_4$, prepared by heating pyrocatechol with para-phenylenediamine. Analogous to phenazine is diamidophenazine, $NH_2 - CH_3$  $C_6H_3 - NH_2$, discovered by Griess,[2] a ruby-red substance which crystallizes in the form of needles. The compound is soluble in hot water; in hot ammonia there is obtained, on cooling, small yellow crystals of the free, almost pure, base. This base dissolves in benzene or alcohol with a green fluorescence, but the alcoholic solution of the salt fluoresces with a dark greenish orange-red color.

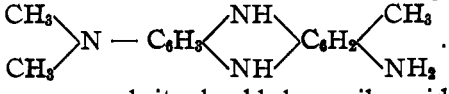
Merz[3] has prepared still another homologue of phenazine, $C_6H_4$  $C_6H_3 - CH_3$, methylphenazine. He found that by the action of ammonium sulfide it could be reduced to the hydro compound, the

[1] *Compt. rend. soc. biol.*, **70**, 514 (1911).
[2] *Ber.*, **5**, 202 (1872).
[3] *Ibid.*, **19**, 725 (1886).

formula of which is  This compound crystallizes in silver-white plates and is easily again oxidized to the original compound. Merz states that the reactions are similar to those of hydrophenazine.

Bernthsen and Sweitzer[1] prepared dimethylaminomethylphenazine,  which is analogous to neutral red but contains one amino group less. They observed that, in contrast to a compound containing less radicals, it is not reduced by ammonium sulfide but only by the prolonged action of tin and hydrochloric acid. The leuco compound arising from the reduction is stated by the authors to be undoubtedly dimethylaminomethylhydrophenazine. It is evident that this compound is very similar to neutral red, which was first prepared by Witt by the action of nitrosodimethylaniline on toluylenediamine. Using *p*-phenylenediamine instead of nitrosodimethylaniline, Bernthsen and Sweitzer also succeeded in obtaining neutral red.

These investigators give the formula for the leuco compound as  which is analogous to the hydro compound of the simple phenazine previously mentioned. They were, however, unable to isolate this substance on account of the great ease with which it oxidized.

It is clear, therefore, that Gage and Phelps were mistaken in their deductions. For, although toluylene blue is an intermediate product in the formation of neutral red, yet it does not necessarily follow that this compound will be formed on gentle reduction. On the contrary, one would naturally conclude that the first reduction product of neutral red would be the hydro compound if one can judge by the behavior of the analogous compounds described above. The formula as given by Bernthsen and Sweitzer is  . From the unstable form of this compound it should be easily oxidized, as are all the hydrophenazines. Theoretically, it would be colorless or nearly so. On account of the numerous groups attached to the benzene rings, it should only be formed from the original neutral red by rather strong reduction. We found, experimentally, that, in accordance with expectation, hydrogen sulfide passed into an ammoniacal solution of neutral red had no effect, although a similar treatment will quickly reduce phenazine. Therefore, one would expect that while some organisms,

[1] *Ann.*, **236**, 332 (1886).

such as typhoid, which will easily reduce methylene blue and similar compounds to the leuco base, their power of reduction is not strong enough to effect neutral red; in fact, as has been repeatedly shown, a strong reducing agent is required to cause a change in this compound. Many bacterial reductions are so strong, however, that there is no question that they are able to cause analogous changes.

In view of the facts recorded above it was thought profitable to undertake a study of the neutral red reaction with the intention of ascertaining, if possible, the nature of the changes produced by the reducing bacteria and to learn the best diagnostic concentration of the components in the Stokes medium.

The investigation was conducted upon the following lines, to ascertain:

1. The influence upon the color change of an increased amount of peptone alone instead of the usual 1 or 2% peptone, together with meat broth or meat extract.

2. The effect of systematic variations of the various components; that is, peptone, lactose, acidity, salt and neutral red on the sensitiveness of the contrast reaction.

3. The effect of these variations on the volume and composition of the gases formed.

4. The true nature of the chemical reactions involved in the color change.

5. The value of neutral red medium as an indicator of fecal pollution, other than human, in drinking water.

## Experimental.

The proper concentrations of peptone, lactose and inorganic salts were first ascertained.[1] Neutral reds obtained from several different firms were tried; no differences were noticed save that slightly different concentrations were required to produce solutions of the same color intensity.

The selection of the fermentation tubes had to be made with care as, in order to obtain a sharp line of demarcation, the neck joining the closed arm and bulb must be constricted to a rather narrow opening. From four to ten fermentation tubes, selected as stated above, were used for each inoculation in order to draw conclusions both as to the uniformity and rapidity of diagnosis. To part of the media the neutral red was added before sterilization and to part, after sterilization in the form of a sterile solution. The incubation was carried on in an ordinary 37.5° incubator.

After preliminary experiments it was found that the neutral red up to 0.02% had no appreciable effect either on the amount of gas or the time of the first evidence of gas production. It was reasonable, therefore, to assume that we could consider the rapidity and volume of gas formation

[1] THIS JOURNAL, 37, 1950 (1915).

entirely independent of the amount of neutral red within the limits mentioned; consequently, if we were to deal with the question of gas production alone, the lactose-peptone medium proposed in our previous paper could be adopted. One fact, however, remained; it was necessary to ascertain if the changes in the various components, even if they had little effect on the gas, might not greatly affect the rapidity of the color change.

The components which might have an influence are: concentration of the neutral red, concentration of peptone, acidity, presence of inorganic salts, and lactose. We, therefore, undertook a study of these in the order named.

**Concentration of Neutral Red.**—A medium was prepared consisting of 3% peptone, 1% potassium chloride, and 0.8% lactose, with an acidity of +1.0%. To portions of this neutral red was added as follows: 0.001%, 0.002%, 0.004%, 0.006%, 0.008%, 0.01%, 0.015%, 0.02%. These eight different concentrations were inoculated with a sewage-polluted water and the conditions maintained as uniform as possible. The experiments showed that all started to change color at the same time; the first three appeared too weak for diagnostic purposes, the second three were of almost the desirable intensity, although the fifth and sixth appeared more nearly the ideal. Above 0.01% neutral red the color change was not as distinct but was a brownish yellow due to too much unchanged neutral red. It was noticed that the change to a fluorescent yellow was gradual, first starting after the evolution of gas had ceased and continuing uniformly through the closed arm. From 0.008% to 0.01% appeared to yield the best results.

It was also found that the peptone could be varied between 2 and 5% without any appreciable effect on the color change. The most satisfactory acidity was found to be +1%, although a difference of 0.1 or 0.2 ± had no noticeable effect.

**Effect of Different Salts.**—In our previous work it had been ascertained that more rapid and more uniform gas production resulted when potassium or sodium chloride was present. In order to see if this held true when neutral red was added, we prepared media, using the following salts in 1% concentrations: KCl, $K_2SO_4$, NaCl, $Na_2SO_4$, $MgCl_2$, $MgSO_4$, $NH_4Cl$, $(NH_4)_2SO_4$, $CaCl_2$. As a result of these experiments it was found that the chlorides of ammonium and calcium retard the color change to a marked degree. On the other hand, the sulfates seem to have very little effect except potassium sulfate and magnesium sulfate, which showed a marked acceleration.

The neutral red medium usually gradually decolorizes on standing and a reddish brown precipitate settles to the bottom of the container. The partially changed medium does not appear to be as sensitive under these

conditions as when no precipitation has occurred. To overcome this difficulty, ordinary lactose-peptone medium was prepared, and, before inoculation, was treated with 0.2 cc. of a 0.4% solution of neutral red (equivalent to a 0.008% solution) and thoroughly mixed. If, however, a more dilute solution is added, allowance must be made in the preparation of the medium. Our results indicated that this method yields a medium somewhat more sensitive than if the dye is present when the medium is sterilized; similar results have been reported by other investigators.

**Effect of the Variation of Lactose.** — Scheffler,[1] using the color change alone as a means of diagnosis, decided that a three-tenths per cent. glucose caused this change to appear in a minimum time. In a previous paper[2] we have shown that, in ordinary media, 0.5% carbohydrate is the least that should be used to obtain a satisfactory gas production. Consequently, if *both* the color change and the gas production are to be employed in the diagnosis, we must have the concentration of the carbohydrate greater than 0.4%.

The following table shows the results obtained by varying the lactose when neutral red is also present. In these experiments, the neutral red was kept constant at 0.008%, the potassium chloride at 1%, and the peptone at 3%:

| Per cent. lactose. | Percentage of tubes showing complete reduction of neutral red to the yellow compound at end of 30 hours. |
|---|---|
| 0.4 | 62.5 |
| 0.6 | 25.0 |
| 0.8 | 33.0 |
| 1.0 | 33.0 |
| 1.5 | 0.0 |
| 2.0 | 0.0 |
| 3.0 | 0.0 |
| 3.5 | 0.0 |

Evidently 1% lactose should be the maximum used when neutral red is employed.

**Experimental Studies upon the Yellow Compound.**—The work of Guerbet[3] was repeated, but we failed to obtain any evidence that when the fermentation was produced by the ordinary intestinal organisms, ammonia entered into the color change. In fact, we could find no evidence of ammonia being formed in any appreciable quantities when the incubated media were tested at the end of thirty-six hours, *i. e.*, immediately after the completion of the color change. Neither could we find any differences, either in the acidity or in the ammonia content,

[1] *Centr. Bakt. Parasitenk, Abt. I*, 38, 199 (1900).

[2] THIS JOURNAL, 37, 1950 (1915).

[3] *Compt. rend. soc. biol.*, 70, 514 (1911).

between the red and yellow solutions when the samples were taken from the same fermentation tube. After we had completed our analyses, Kendall, Day and Walker,[1] using glucose broth instead of lactose in proteolytic studies, obtained results entirely confirming our conclusions. Of thirteen strains of *B. coli* studied, they found none gave an increase in ammonia after two days and only seven at the end of seven days. In the latter strains this amount was in nearly every instance very slight. They conclude that in the presence of carbohydrates the proteolytic action of *B. coli* is very slight.

Guerbet states that the turbidity of the culture is one of the causes of its fluorescence, but makes no mention of experimental work in proof of this. In order to test the validity of his theory it was deemed essential to experiment with both the yellow fluorescent compound produced by bacteria and also with the yellow compound formed by chemical means. A study of the action of various common reducing agents was first made. Of these, tin and hydrochloric acid gave the yellow compound but the reaction was slow and unsatisfactory. Sodium hydrosulfite[2] was next employed. A small quantity, either in the form of an aqueous solution or a dry powder, if added to a water solution of neutral red, will cause the red color to change to a light yellow which is slightly fluorescent. On addition of suspended matter to this yellow solution the fluorescence is much more pronounced.

To determine if the suspended matter present in the regular lactose-peptone medium has the same effect on the fluorescence, some of the yellow incubated medium was taken from the closed arm, and filtered through a very fine Berkefeld filter which removed all except the colloidal particle. The liquid was then tested for fluorescence by illuminating the solution with light of one color obtained from a slit and a large prism. The following results were obtained:

| Spectrum color. | Appearance in liquid. |
|---|---|
| Red | No light |
| Green | Green |
| Blue | Green |
| Violet | Green |

It is evident, therefore, that the yellow-green compound is truly fluorescent. We noticed, however, that the fluorescence was less marked than in the turbid liquid and conclude that undoubtedly the turbidity of the culture increases the fluorescent appearance.

We next tried the effect of sodium hyposulfite on some of the port-wine colored medium from the open arms of a fermentation tube after an incubation period of from thirty to forty hours. The port-wine color was immediately changed to a fluorescent greenish yellow identical in

[1] THIS JOURNAL, 35, 1201 (1913).

[2] $Na_2S_2O_4$.

color with the one produced by the bacteria. Indeed, it was impossible to distinguish between the two products when placed side by side. The yellow color thus prepared changed back to the red again and dyed wool in a similar manner to the bacterial product.

An effort was made to isolate the yellow synthetic compound from an aqueous solution. According to statements in the literature concerning analogous compounds, this product should be very unstable and easily oxidized. Experiments proved this to be the case. It was easy to obtain the base by the use of sodium hydrosulfite in alkaline solution, but every attempt to obtain it in the pure dry state failed. The solution was evaporated in a current of specially purified hydrogen and also in a current of carbon dioxide but as soon as all the water had been removed, the yellow mass changed color and when complete dryness was reached became red. The affinity this yellow compound has for oxygen is very remarkable. Thus far we have found no method of removing it from the tube or handling in any way for analysis; the slightest trace of oxygen converts it at once into neutral red.

When attempts were made to extract the color with immiscible solvents, the oxidation took place immediately even if the solvent had been recently boiled to drive out all the air. It appeared, therefore, that the compound is oxidized by the least trace of oxygen, even when evaporation is carried on at a very low temperature. We could do even less with the compound produced by the reducing action of bacteria.

A microscopic study of the substances gave the following results: The neutral red base crystallizes in long needles, the majority of which show parallel extinction and probably belong to the monoclinic system. Neutral red in aqueous solution, when reduced by alkaline hydrosulfite, shows botryoidal masses but no definite crystalline form and rapidly oxidizes to the neutral red base, yielding the characteristic acicular crystals. When the yellow compound obtained by the action of bacteria was allowed to stand in the air until red, and the coloring matter extracted with ether, allowed to evaporate and then taken up with water in which had been placed a little ammonium hydroxide, characteristic red needles of the free base separated. These red needles have all the optical properties and give all the tests of neutral red. The evaporated yellow solution gives the same reddish yellow botryoidal masses obtained from neutral red by reduction.

In conclusion, it can only be said that the yellow compound is probably dimethyldiaminomethylhydrophenazine. We have not as yet been able to prove this save that it yields neutral red on oxidation and must offer it as a very likely assumption based upon chemical microscopic reactions.

Certain it is that the yellow compound produced by the reduction of neu-

tral red base which is conceded to be $N(CH_3)_2—C_6H_3\langle^{NH}_{NH}\rangle C_6H_2(CH_3)—NH_2$ (dimethyldiaminomethylhydrophenazine) differs in no way that we have been able to ascertain from the yellow compound produced by the reducing action of members of the *B. coli* group upon neutral red.

**Diagnostic Value of the Neutral Red Medium.**—It has been fully demonstrated by various experimenters referred to above[1] and also by long experience in our laboratory that the neutral red medium is a very sensitive indicator of the presence of animal fecal matter. In fact, it is our belief that the medium is much more sensitive than the standard lactose-bile medium. A few experiments were tried with a view of forming conclusions as to its value for diagnosing contamination produced by the feces of domestic animals.

Samples of the feces of the following animals were collected from the University Stock Farm: Horse, two samples; cow, two samples; sheep, one sample; calf, one sample; pig, two samples.

Contaminated waters were prepared as already described in our study of the lactose-peptone medium and lactose neutral red fermentation tubes inoculated therewith. The results showed conclusively that the medium is a good indicator of pollution by fecal matter. The final gas volume was between 50 and 60% and the characteristic contrast reaction was always positive. In all respects the reaction is similar to that obtained with human feces or sewage.

## Conclusions.

1. A neutral red medium composed of from 3–4% peptone, 0.8% potassium chloride or potassium sulfate, 0.6% lactose, 0.008% neutral red with a reaction of +1% affords a very sensitive and accurate medium for the speedy detection of fecel pollution by bacteria. The addition of meat broth increases the sensitiveness of the medium but is not essential.

2. The yellow fluorescent compound formed by the action of the bacteria is probably dimethyldiaminomethylhydrophenazine, a simple re-

[1] Cf. also Ris, "Ueber das phenazin," *Ber.*, **19**, 2206 (1886); Rambousek, "Vergleichende und kritische Studien betreffend die Diagnostik des *Bact. typhi* und des *Bact. coli*," *Arch. Hyg.*, **38**, 383 (1900); W. Hunter, "A Method of Distinguishing *Bacillus coli communis* by the Use of Neutral Red," *Lancet*, **1**, 613 (1901); R. H. Makgill, "The Neutral Red Reaction as a Means of Detecting *Bacillus coli* in Water Supplies," *J. Hyg.*, **1**, 430 (1901); W. G. Savage, "Neutral Red in the Routine Bacteriological Examination of Water," *Ibid.*, **1**, 437 (1901); A. Oldekpp, "Eine Modification des Rothberger schefflerschen Neutral Rot Nahrbodens," *Centr. Bakt. Parasitenk, Abt. I*, **35**, 120 (1903); Braun, "Le rouge neutre et le diagnostic rapide de la souillure des eaux de boisson par le coli bacille," *Bull. inst. Pasteur.*, **4**, 561 (1906); A. McConkey, "Lactose Fermenting Bacteria," *J. Hyg.*, **9**, 89 (1909); A. Sicre, "Au subject du rouge neutre comme indice du coli bacille," *Compt. rend soc. biol.*, 66, 152 (1909).

duction product of neutral red. Ammonia does not enter into the formation of the reduction product.

3. The Stokes neutral red medium is a convenient and reliable one for the detection of fecal contamination in water and is more sensitive than lactose bile.

NOTE.—It is unfortunate that, owing to the loss of a large amount of data in the burning of the Cornell University chemical laboratory, we cannot give a summary of the results obtained on a very large number of samples covering a period of five years. It is on a careful study of these that we have based our third conclusion. Nor are we able to give the data obtained with "synthetic media" bearing on our conclusions under II. This paper is presented in its unfinished condition since it would require several years more of work to again obtain data.

ITHACA, N. Y.

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[CONTRIBUTION FROM THE LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

# ON THE COMPOSITION AND DIGESTIVE ACTIVITY OF DIFFERENT FRACTIONS OF THE PANCREAS. II.

BY R. A. NELSON AND J. H. LONG.

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Several papers have appeared from this laboratory on the subject of the behavior of fractions of the pancreas of certain domestic animals, secured by aid of strong centrifugal action on the finely minced gland. In the second of these,[1] by one of us and others, it was shown that the ferment activity is not uniform in different layers of the centrifugal mass and further that this activity differs greatly from one animal to another. Not much was said about the fat-splitting function of the different fractions, that property being reserved for fuller study. It was shown that the amylopsin value is very marked only in case of the hog pancreas, while for the beef and sheep organs it appears to be low.

When the minced pancreas is packed into centrifuge tubes and rapidly rotated, about 3000 revs. per min., the mass separates into three layers. When the tube comes to rest the top layer is found to contain a large amount of fat, the middle layer is a liquid which may be easily filtered, while the lower layer contains mostly protein. To effect a good separation the temperature of the centrifuge must get high enough to partially melt the pancreas fat, which, however, is accomplished without difficulty, as this is a very soft fat. The amylopsin is readily soluble and collects mainly in the liquid layer, but that is not the case with the other ferments. It was found, for example, that in testing the liquid layers from four hog pancreases, and the top and bottom layers under the same conditions, the following values were obtained. One gram of substance from each

[1] THIS JOURNAL, 37, 2427 (1915).