

A NOTE ON THE NATURE OF THE REACTION OF *B. COLI* ON ENDO MEDIUM

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There appears to exist much difference of opinion as to the factor or factors which enter into the production of typical colonies of *B. coli* on the Endo plate. Endo (1904), who developed this medium for the differentiation of *B. typhosus* from *B. coli*, states that the red colonies are due to acid produced by the *B. coli*.

Harding and Ostenberg (1912) claim that the red colonies of these organisms on the Endo plate are due to aldehyd formation, and that the gradual disappearance of the color after the first twenty-four to forty-eight hours incubation, is due to oxidation of aldehyd to acid. According to these workers, acid decolorizes Endo medium. De Bord (1917), on the other hand, claims that acid is essential for the production of red colonies; that aldehyds will not bring out the red color in the Endo medium, but acid and aldehyd will. This worker, like Levine, Weldin, and Johnson (1917) speaks of the Endo reaction as the "fuchsin-aldehyd reaction," insisting however that acid is essential for this reaction.

According to Robinson and Rettger (1916), organic acid, especially lactic acid, is the cause of the reddening of colon colonies on Endo medium, and they maintain that the later decolorization of the colonies, is due to alkali formation by the bacteria. These investigators find that a drop of lactic acid added to an Endo plate, produces a shade of red somewhat similar to that produced by *B. coli*. On the other hand, a drop of neutral

formaldehyd added to the Endo medium produces a purple-violet color.

The underlying cause of the different views expressed by these investigators, appears to be due to the variation in the strength of the reagents employed in their respective experiments. It has been observed again and again that fuchsin decolorized with sodium sulfite will behave differently toward different dilutions of the same reagent. The behavior of the fuchsin sodium sulfite solution toward strong and weak acid, or strong and weak aldehyd is such that it can not be compared. Furthermore, the quantity of sodium sulfite employed in the decolorization of the fuchsin also appears to play an important part in such experiments.

The fuchsin-sulfite combination is extremely unstable. Workers have long learned not to expect complete decolorization of the fuchsin with sodium sulfite in hot solutions, because of the dissociation under these conditions. When an Endo plate is exposed to air, the color of the medium becomes pink, probably because the sulfite in the presence of air is oxidized to sulfate, causing the fuchsin color to reappear in part. Very dilute acids also bring out the color to some extent, possibly because of the high degree of dissociation that exists in the mixture.

If inorganic acids of moderate strength be added to fuchsin decolorized with sodium sulfite, decolorization becomes even more complete. The sodium probably combines with the acid to form a salt, liberating sulfur dioxide or sulfurous acid, and causing further decolorization. Concentrated acids will reduce the color of basic fuchsin without the presence of sodium sulfite.

Organic acid added to decolorized fuchsin will cause a reappearance of the color, due possibly to a stronger affinity of basic fuchsin for the organic acid than for the inorganic sulfite, the result being the formation of acid fuchsin.

It might be said in this connection that we are dealing here with an extremely complex organic combination, and it is questionable to what extent one is permitted to draw a conclusion from a simple test tube experiment. The following few observations will be recorded with the hope that they may throw

some light on the original problem, of the cause of the red colonies on the Endo plate.

When strong aldehyd is added to decolorized fuchsin, a strong purple color is produced. Weak aldehyd produces a red color; weak aldehyd in an acid solution, a strong cherry red color. If a proper combination of aldehyd and acid be added to the decolorized fuchsin in a test tube, a metallic film will appear on the surface in a few minutes. This film, however, is not permanent, disappearing after a few hours. Acetone and alcohol in faintly acid solutions will also bring back the color to decolorized fuchsin. This, it might be added, is a matter of text-book knowledge.

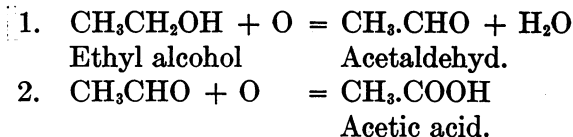
It was further observed that, if glucose be substituted for lactose in Endo medium, not only are typically metallic colonies produced by the colon group, but by the typhoid-dysentery group as well. The same was also found to be true when the triatomic alcohol, glycerol, and the hexatomic alcohol, mannitol, were substituted for lactose in Endo medium.

Finally, the following experiments were performed. Two 1000 cc. Erlenmeyer flasks containing 300 cc. of 1 per cent lactose broth, and 1 per cent glycerol broth respectively, were inoculated with a fresh agar slant growth of *B. coli-communior* obtained from feces. These flasks were employed in order to permit large surface exposure. After twenty-four hours' incubation the contents of the flasks were rendered alkaline with sodium carbonate to hold back the organic acids, and distilled.

When the distillates were added to decolorized fuchsin, no change could be observed. When, however, the distillates were rendered faintly acid with acetic acid and added to decolorized fuchsin, the appearance of the color was far more marked than when the same concentration of acetic acid alone was employed. This would indicate that another substance, or possibly other substances, besides acid, are formed, when *B. coli* is grown under partial anaerobic conditions in lactose or glycerol medium, which help to bring back the color to decolorized fuchsin.

In this connection, it might be well to review briefly the chemical nature of some of the substances under discussion.

(1) The close relation which exists between alcohol, aldehyd, and acid is well illustrated by the following equations. If we take ethyl alcohol as our specific example, we have:

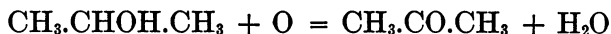


Thus, by oxidation, Alcohol \rightarrow Aldehyd \rightarrow Acid
and inversely by reduction Acid \rightarrow Aldehyd \rightarrow Alcohol.

It is generally believed that oxygen from the air can not effect the oxidation from alcohol to acid except through the agency of "catalyzers" or ferments.

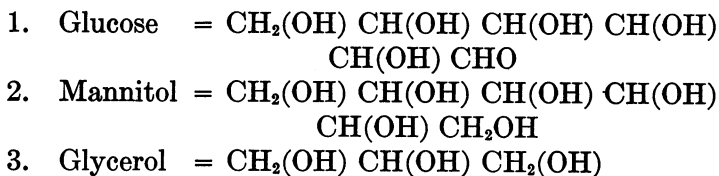
(2) The close relation which exists between aldehydes and ketones can be seen from the following:

The oxidation of primary alcohols yields aldehydes, and the oxidation of secondary alcohols yields ketones—thus



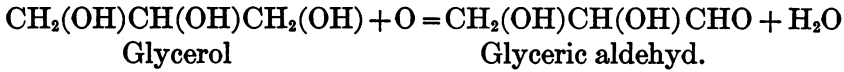
Secondary propyl alcohol Dimethyl ketone or acetone.

(3) From a chemical standpoint, the carbohydrates are aldehyd or ketone derivatives of polyhydric alcohols. This is illustrated by glancing at the structural formula of a typical sugar, glucose, and the two alcohols, mannitol and glycerol.



(4) The production of lactic acid from lactose by a number of organisms, including *B. coli*, has led some investigators to the opinion that the reddening of colonies produced by *B. coli* on Endo medium is due to lactic acid. It is indeed likely that the formation of lactic acid is an important intermediary step in the production of typically metallic colonies on Endo agar. However, when the alcohol, glycerol, is substituted for lactose in

Endo medium, the explanation of typical colonies produced on this medium becomes more complex. It is likely that the glycerol is first oxidized into glyceric aldehyd,



and this in turn is converted into lactic acid. It will be recalled that Embden and his co-workers (Hammarsten, 1914) have shown that in the formation of lactic acid from glucose by enzymes, glyceric aldehyd is one of the intermediary products.

Grey (1913), who has investigated the products of anaerobic glucose decomposition by *B. coli-communis*, finds that this organism produces lactic, acetic, formic, and succinic acids, alcohol, and acetaldehyd from this carbohydrate. Mendel (1911) finds also the presence of acetone among glucose decomposition products of *B. coli*.

To recapitulate the observations discussed above:

1. Acid, aldehyd, acetone and alcohol in proper dilution and combination cause a reappearance of the fuchsin color of a decolorized fuchsin-sulfite solution.

2. When glucose, mannitol and glycerol are substituted for lactose in the Endo medium, typically metallic colonies are produced by the entire typhoid-colon group.

3. *B. coli-communior*, when grown in lactose and glycerol broth under partially anaerobic conditions, produces another substance—or possibly substances—besides acid, which bring back the color to decolorized fuchsin, when faintly acidified.

In view of these observations and those of the workers mentioned above, it would appear that the red colonies which *B. coli* produces on the Endo plate are due to a number of substances—including lactic and other organic acids, traces of aldehyd and possibly also acetone and alcohol. The latter of these substances are volatile and therefore evaporate on exposure, the result being that the non-volatile fuchsin remains behind and we thus have the metallic film.

The possible criticism that *B. coli* breaks down glucose into the various substances named above, only when grown anaerobi-

cally, but not during the aerobic growth on Endo agar, does not appear to hold. Organisms growing on the surface of agar must indeed have the ability to grow in the presence of air in order to get a start, so to speak. But once the start is gained the surface organisms alone grow aerobically, while those organisms below the surface of the colony are probably growing in no less an anaerobic environment than those growing below the surface in carbohydrate broth.

Observations of the gradual disappearance of the metallic film of colon colonies on Endo agar corroborates this view. It is reasonable to assume that the organisms below the metallic film of a colon colony, are growing in an anaerobic environment. These organisms, after twenty-four to forty-eight hours incubation, proceed to break down the nitrogenous bodies of the nutrient media into ammonia and amines (Robinson and Rettger), creating a condition favorable for the re-solution of the metallic fuchsin and final decolorization. In other words, we have here a chemical change produced by *B. coli* growing *anaerobically* in apparently aerobic colonies.

As further evidence that the metallic sheen of colon colonies on Endo medium results from the evaporation of volatile substances produced during the metabolism of these organisms, the following two observations are cited.

1. If *colon bacilli* are grown anaerobically in Endo agar, the colonies are red but not metallic. Under anaerobic conditions, *B. coli* breaks down carbohydrate into various acids, aldehyd, and possibly acetone and alcohol (Grey and Mendel). These substances bring out the color of decolorized fuchsin and the colonies become red. The fuchsin, however, remains in solution, because the volatile substances can not evaporate. The same colonies exposed to air become metallic, because surface evaporation permits the disappearance of the substances which hold the fuchsin in solution.

2. The metallic sheen of a given colon colony is markedly pronounced when grown in such a manner that evaporation can take place on a large scale. Thus, if *B. coli* be inoculated on Endo plates and on Endo agar slants, the metallic film, after a

given period of incubation, will be more pronounced on the Endo agar slant than on the Endo plate. This is explained as due to the greater opportunity for evaporation from the slant than from the inverted plate.

There are a large number of red colonies on Endo medium which workers recognize to be of non-colon types. These colonies, as a rule, possess a red shade that is somewhat different from that of *B. coli* and they never possess a metallic film. It is possible that some organisms cause the production of red colonies by reducing sodium sulfite to hydrogen sulfide, thus liberating the fuchsin color. It is more likely, however, in view of the ease with which bacteria attack carbohydrates, that these red colonies are produced by the conversion of lactose to some non-volatile acid, most probably lactic acid. This acid has a tendency to absorb moisture from the air, and such moisture on the surface of the colony would tend to keep the fuchsin in solution, rendering the colony red but not metallic.

CONCLUSIONS

1. Chemical and physical factors enter into the formation of typically metallic colonies of *B. coli* on Endo agar.
2. During the growth of these organisms on Endo medium, they adsorb the various soluble substances contained in the medium and proceed to break down the lactose first (Kendall).
3. After ten to fifteen hours incubation, the trace of lactose adsorbed, is probably transformed into lactic and other organic acids, and the colony is colored red.
4. On further incubation, the organic acids are probably reduced by the bacteria to aldehyd and alcohol, which volatilize from the surface, leaving the non-volatile fuchsin behind, thus producing a metallic sheen.
5. The carbohydrate being disposed of, the organisms proceed to attack the nitrogenous materials. Ammonia and other substances are produced in which the fuchsin goes back into solution, and ultimate decolorization takes place.

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