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Inactivation of Nutrients by Heating With Glucose[1]

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Hill and Patton (*1*) found that the slight discoloration occurring during the autoclaving of media for the microbiological assay for L-tryptophan was caused by interaction with glucose, resulting in decreased growth of *Streptococcus faecalis* R. It was not known at that time whether the poorer growth resulted from destruction of nutrients or formation of growth inhibitors as products of the browning reaction. The work reported here indicates that the decreased growth is due to destruction of nutrients.

TABLE 1

LOSS OF L-TRYPTOPHAN DUE TO INTERACTION WITH GLUCOSE

Treatment	Browning (% T/610)	Hydroxymethyl-furfural (%)	Tryptophan loss (%)
Unheated	100	0	0
Heated	81.5	0.52	60
Heated at pH 10 ..	41.5	2.25	26

It is known that the browning reaction is promoted by alkalinity, and that hydroxymethylfurfural is one of the chief reaction products. Advantage was taken of these facts in the following tests: Aliquots of a solution containing known amounts of L-tryptophan and D-glucose were heated under suitable conditions to cause browning similar in appearance to that which occurred during autoclaving of media. The extent of browning was measured by determining the decrease in transmission at 610 mμ in a Coleman spectrophotometer. Hydroxymethylfurfural content, used as an indicator of the concentration of browning reaction products, was estimated from the absorption increase at 284 mμ in a Beckman DU spectrophotometer. The loss of L-tryptophan resulting from the browning reaction was determined by microbiological assay using the sucrose medium to prevent further browning loss. Aliquots buffered at pH 10 were prepared to obtain samples in which browning was only partially due to interaction with tryptophan. These mixtures produced more browning in less time. Growth of *Str. faecalis* R was measured turbidimetrically after 16 hrs by decrease in transmission at 610 mμ. At the dilutions used for assay, the browned samples were colorless at this wave length.

As shown in Table 1, better growth was obtained from the tryptophan-glucose sample heated at pH 10, in spite of the fact that more browning occurred and more hydroxymethylfurfural was formed. On the other hand, the solution containing only glucose and tryptophan, showing less browning and much less hydroxymethylfurfural formation although heated for a longer time, permitted poorer growth. These data indicate that decrease in growth was due not to formation of growth inhibitors as products of the browning reaction but to actual destruction of part of the tryptophan.

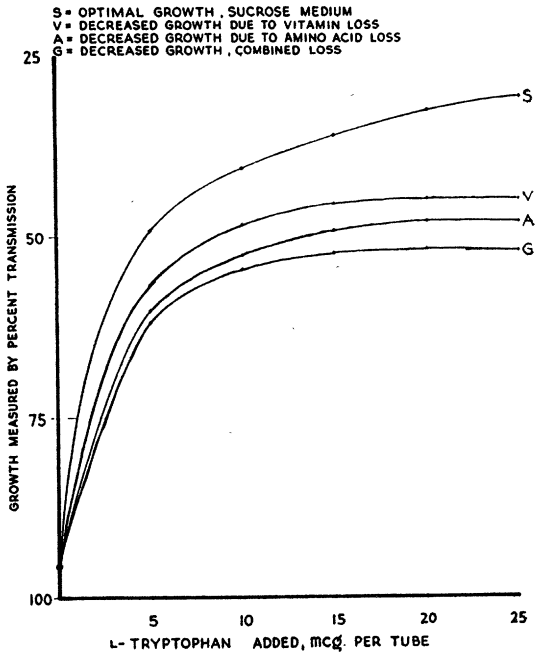

FIG. 1. Growth of *Str. faecalis* R in standard series for L-tryptophan assay, showing decreased growth due to autoclaving in presence of glucose.

It also appears that such destruction is not limited to tryptophan. Both L-lysine and DL-methionine, upon heating in the presence of glucose, underwent similar destruction, as determined by subsequent microbiological assay using synthetic amino acid media. Curve G (Fig. 1), which is a standard growth curve for L-tryptophan as obtained by the customary assay method using glucose in the medium, shows the total growth-decreasing effect of the browning reaction. Curve V resulted from the addition of a pure sterile solution of the amino acids to the autoclaved medium before inoculating, in an attempt to reveal the extent of vitamin destruction during autoclaving. Similarly, to produce curve A the vitamins were replaced after autoclaving, to show the extent of amino acid destruction. These curves indicate that nutrients in both the vitamin class and the amino acid class suffered

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damage during autoclaving with glucose. The vitamins required in the medium (B complex) contain nitrogenous moieties which might conceivably react with glucose similarly to amino acids. Inactivation of nutrients was minimized by using sucrose instead of glucose in the medium (Curve S), as previously described by the authors (*1*).

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Effect of Suspended Silt and Other Substances on Rate of Feeding of Oysters

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Our recent studies on the feeding of oysters (*Ostrea virginica*) with cultures of *Chlorella* sp., *Nitzschia closterium*, *Euglena viridis*, and other forms have shown that they feed most efficiently when the numbers of food microorganisms in the water are relatively small (*2*, *3*). These conclusions are in agreement with the theoretical discussions of Kellogg (*1*) and Yonge (*5*). We found that both the filtrate of the cultures containing metabolic products of the cells and the cells themselves affected the rate of oyster feeding.

In continuing our studies on the feeding of oysters we substituted for living cells various turbidity-creating substances, such as fine silt collected from the tidal flats of Milford Harbor, a clay-like substance—kaolin (aluminum silicate), powdered chalk, and Fuller's earth. All these substances may sometimes be found under natural conditions in suspension in inshore waters. Silt, which is a mixture of organic and inorganic substances, is, of course, very common and always present in varying quantities in inshore waters inhabited by oysters.

The methods employed were virtually the same as those used in our earlier studies on the effect of different concentrations of microorganisms upon oysters (*3*). They consisted chiefly in recording, on the kymograph under normal conditions and when water was rendered turbid in various degrees, the rate at which the oysters pumped water through the gills and the changes in their shell movements. The concentrations ranged from 0.1 to 4.0 gm of turbidity-creating substances/l of water, except in the case of Fuller's earth, where only one concentration of 0.5 gm/l was used.

In the first series of experiments the water was made turbid by the addition of silt. Even when small quantities, such as 0.1 gm/l, were added, the type of oyster shell movement changed and the rate of pumping considerably decreased. The reduction averaged 57% (Fig. 1), but in some individuals the rate of pumping was decreased 87%.

A sharp decrease in the rate of pumping was always noticed when concentrations of 0.25, 0.5, 1.0, 2.0, and 4.0 gm/l of sea water were made. In concentrations of 1.0 gm of silt/l, the average rate of pumping decreased more than 80%, reaching a decrease of about 94% in concentrations of 3 and 4 gm/l. Although such heavy concentrations seldom occur in nature, they may, nevertheless, arise during heavy floods or be created in areas where the bottom deposits are artificially disturbed, as happens in the case of channel dredging.

The results of the experiments with kaolin and chalk were similar to those obtained with silt (Fig. 1). The addition of even such small quantities of these substances as 0.1 gm/l noticeably decreased the rate of pumping. This became more evident as the concentrations were increased. Nevertheless, even in high concentrations, the

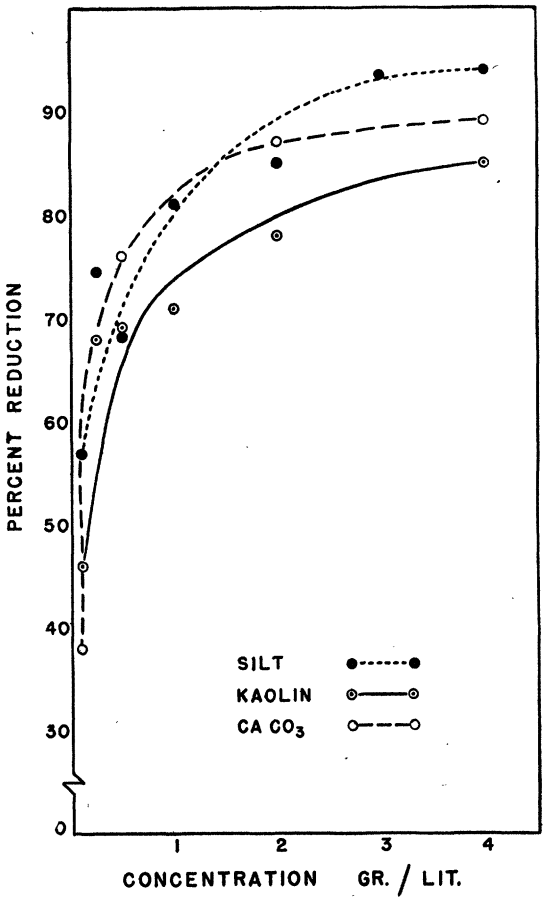

FIG. 1. Per cent reduction in pumping rate of oysters subjected to different concentrations of turbidity-creating substances.

majority of the oysters kept their shells open most of the time and pumped some water. Fuller's earth was used only in one concentration, 0.5 gm/l, reducing the rate of pumping by 60%.

It is possible that in the case of chalk the depression of the rate of flow was in part due to the chemical action of that substance. This possibility is now being investigated, and the results will be reported in the final article.

The oysters expelled, in the form of pseudo-feces, large quantities of suspended materials. Nevertheless, some