of particulate or organic materials, the quaternary ammonium compounds probably do disinfect.

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The Maillard Reaction in Microbiological Assay[1]

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In 1912 Maillard (*1*) pointed out that solutions containing amino acids and reducing sugars turn brown when heated. Although this browning reaction has recently been recognized to be of the greatest importance in nearly all industries concerned with materials of biological origin, including foods, it is still little understood because of its complexity. It occurred to the writers that further insight into the Maillard reaction might be gained through use of the microbiological assay technique, since the latter involves the autoclaving of solutions containing amino acids and dextrose (a reducing sugar) under conditions favorable to the Maillard reaction.

Accordingly, the customary casein hydrolysate medium was prepared for tryptophane assay with *Streptococcus faecalis* in such a way that the reducing sugar component of the medium (dextrose) could be autoclaved separately from the rest of the medium for comparison with a "normal" series, using medium containing identical ingredients but autoclaved in the presence of dextrose in the customary manner.

The growth curves obtained are shown in Fig. 1. Good growth was obtained by the standard procedure (Curve A), but still better growth was obtained in the medium prepared by autoclaving the dextrose separately from the rest of the medium and combining aseptically after cooling (Curve B). These results have been repeated and are reproducible.

It thus appears likely that optimal growth may seldom be reached in microbiological assays, due to the effects of the Maillard reaction. This would not be discovered in recovery tests, since both standard and unknown series would be autoclaved alike. The question arises, however, whether this apparent accuracy of the microbiological assay can always be relied upon, in view of the possibility that standard and unknown samples might differ in their ability to alter the influence of the Maillard reaction upon growth.

Since sucrose is not a reducing sugar and does not readily take part in the Maillard reaction, it was thought that sucrose might be preferable to dextrose, if satisfactory growth were obtained in a medium containing sucrose. Accordingly, a third series was prepared and run simultaneously with the assays shown in Fig. 1. In this C. P. sucrose replaced the dextrose ordinarily used but at one-half equimolar concentration (final concentration of sucrose in the medium, 1/12 M). Here again the growth obtained was better than "normal"; in fact, the growth curve obtained with sucrose in the medium was identical with Curve B (Fig. 1).

A certain amount of browning during autoclaving is commonly accepted as being inevitable in microbiological assay;

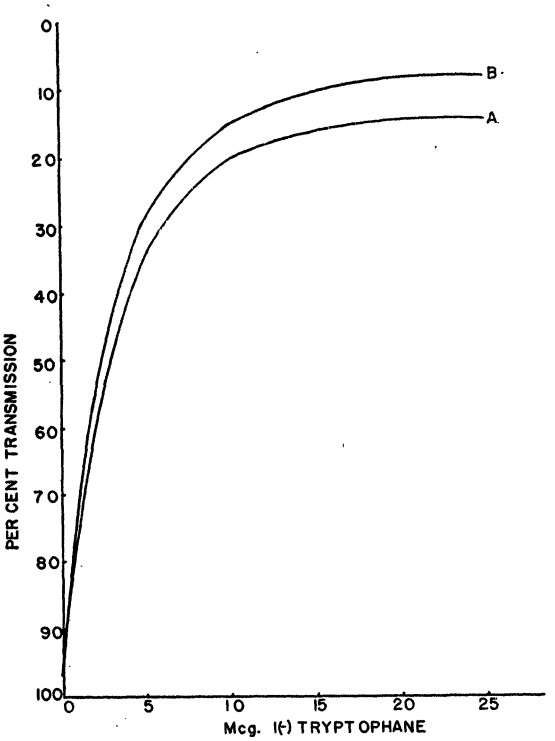

Fig. 1. Growth curves for tryptophane assay using *Str. faecalis*.

however, our media using either sucrose autoclaved with the medium or dextrose autoclaved separately were *water white*. Compared with these, the normal medium transmitted 84.5 per cent as much light at 610 mμ, the decrease in transmission being due to the browning which occurred during autoclaving.

Whether the poorer growth obtained by the customary procedure is due to destruction of nutrients or production of growth inhibitors has not yet been determined. The Maillard reaction produces an increase in acidity, and the possibility that this may be responsible for the reduced growth has not been overlooked. The fact that the greatest differential in growth occurs at the higher growth levels lends support to this possibility. The actual difference in acidity due to browning was rather slight, however, amounting to a difference of only 0.96 ml. of 0.1 N acid in the uninoculated blank tubes, which was responsible for a difference in pH of only 0.2 (Curve A medium, pH 6.96; Curve B medium, pH 7.16).

The possibility that these results might be a peculiarity of the tryptophane assay, due to destruction of tryptophane during autoclaving, was ruled out by another series of assays. In these, graded levels of l-tryptophane solution were autoclaved *separately* before adding to the autoclaved media.

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Again, the same results were observed: the greater the extent of browning which occurred during autoclaving, the less the subsequent growth obtained with a given level of tryptophane.

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A Simple Method for Rearing Blowflies Without Meat[1]

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The methods used for rearing blowflies to be used in experiments on insect physiology or toxicology are reviewed by Woodbury (*2*). The disadvantages of these methods, in all of which meat or fish are used for larval food, are twofold: (1) Meat and fish are not always readily or cheaply obtainable, and (2) rotting of the flesh during larval feeding results in unpleasant odors.

Many expedients have been tried to minimize the escape of odors, but none of these is entirely satisfactory. Recently Hill, Bell, and Chadwick (*1*) have solved the problem by rearing blowflies on a sterile synthetic medium. While this method does eliminate most of the odors and allows control of the larval food, it involves the compounding and autoclaving of the medium. These procedures are time consuming and may even be impossible without technical assistance. Accordingly, a simple method for rearing blowflies in quantities, without using putrid meat, seemed desirable. *Phormia regina* Meigen was used in most of the tests, but other species of blowflies could undoubtedly be reared as well.

In the method developed the medium used for rearing larvae is ordinary commercial kibbled dog biscuit.[2] The bone-shaped biscuit can also be used but must be broken into small pieces.

Rearing containers are one- and two-quart Mason jars or gallon mayonnaise jars. In a jar, dog biscuit to a depth of about two or three inches is covered with water and allowed to stand for about two minutes. The water is then poured off, and eggs or young larvae of the blowflies are added. The jar is capped with a circle of fine-mesh wire cloth held in place by a ring cap. This allows air to enter freely and excess moisture to escape, while preventing the escape of the larvae. The larvae generally do considerable migrating, both in the mass of dog biscuit and on the walls of the jar.

When the larvae are nearly full grown, the jar is almost filled with coarse sawdust. In this, the larvae crawl about and pupate. The sawdust and pupae are then removed and the pupae sorted out for emergence, or the sawdust is left in the jar and the flies allowed to emerge there. Flies are removed from the jar by attraction to a light source.

Correct moisture content of the larval medium is an important consideration. This depends on the particle size: the smaller the particles, the less water needed for optimum moistness. Since different brands of dog biscuit and even different samples of the same brand vary considerably in particle size, the technique of moistening the material must be acquired by experience. If the medium is too moist, the larvae leave the food quite early and crawl about in the film of water on the inside of the bottle. In this case it is necessary to add a layer of sawdust, about one inch deep, over the dog biscuit. This usually takes up the moisture and drives the larvae back to the food.

If the dog biscuit is relatively coarse, the larvae will crawl about in it freely to a depth of three to four inches. If, on the other hand, the dog biscuit is fine, the larvae are restricted to the topmost layer and go only about one inch deep. Since their feeding ultimately causes the medium to pack, whatever its original consistency, the dog biscuit is utilized only to a depth of about two inches. Thus, increased production of flies cannot be obtained by increasing the depth, but only by using more jars or jars of larger diameter.

The production of large, well-formed flies is obviously dependent upon the density of larval population. Thirty to 40 grams of dog biscuit will produce 100 such flies. In a two-quart Mason jar we use about 250 grams of dry dog biscuit, and this produces up to 1,000 flies. Under these conditions, 100 flies are produced at a cost of about $.01, using dog biscuit purchased at a retail price of $.60–$.70 for 5 pounds. If purchased in bulk at wholesale rates, the cost would obviously be much lower.

Adult blowflies are kept in screen cages, where they are supplied with water, sugar, and moistened dog biscuit. The latter is both the oviposition medium and protein food necessary for oviposition. Since blowflies usually will not oviposit on freshly moistened dog biscuit, this material is allowed to remain moist a day or two prior to its use in the cages. If the atmosphere is dry, the dog biscuit is kept damp in the cages by placing it in a finger bowl lined with paper toweling on which a bottle of water is inverted.

Whether blowflies reared on meat will oviposit on moistened dog biscuit has not been determined. In earlier work, the eggs were collected on meat and transferred to dog biscuit for rearing only; thus, when the latter was tested for oviposition, it was tried with flies reared on it. Also tested for oviposition were nutrient agar and filter paper soaked in a strong nutrient broth kept moist by inverting a bottle of water on it. Blowflies fed on both of these but oviposited only on the filter paper. The number of eggs laid on the filter paper, however, was not great enough to warrant continuation of the tests.

This method for rearing blowflies makes possible the production of numbers of large flies with little apparatus and expense. Strangely, the moistened dog biscuit does not develop any objectionable odors while the larvae are feeding, the total lack of ammonia being particularly surprising. Odors that develop during the pupal period are held in the bottles by the sawdust. Thus, the rearing containers may be kept in an office or laboratory, no provisions being needed for air conditioning. Since no autoclaving or special preparation of media are needed, the method is very simple, and the relative constancy of composition of dog biscuit assures that the flies are physiologically quite uniform.

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[1] The technical assistance of Mr. Arnold Hultquist in testing this method is gratefully acknowledged.

[2] Brands used successfully in this laboratory are Milk Bone (National Biscuit Company), Daily (The Great Atlantic and Pacific Tea Company), and Kibbies (Morton's Dog Food Company, Minneapolis).