marked accelerating effect on the reduction of TTC.

This paper (*3*) reports the applicability of TTC, NT, and BT for the testing of milk under different conditions. The tetrazolium tests were performed simultaneously with a conventional methylene blue test in unstoppered glass tubes. The final concentrations of the tetrazolium salts were 10^{-3} and 10^{-4} (9 ml of milk and 1 ml of 1.1-percent weight per volume stock solutions of TTC, NT, and BT).

The incubation temperatures were 37°C and room temperature was 19° to 21°C. At room temperature, the tests were performed simultaneously in darkness and under the influence of scattered light of blue sky or of direct sunlight. The bacterial contents of the milk samples were estimated customarily by counting the number of the colonies on agar and gelatin plates.

Tetrazolium salts in a concentration of 10^{-3} were inhibitory to the milk bacteria since the reduction times were definitely prolonged. The concentration 10^{-4} was, therefore, more suitable for the testing of milk. The results obtained using a 10^{-4} concentration of the tetrazolium salts and a dark thermostat of 37°C are summarized in Table 1.

The table shows that the reduction of the tetrazolium salts in the milks with 4×10^6 bacteria per milliliter or less becomes visible earlier than the reduction of methylene blue. In good milks with long reduction times the colors of formazans are first seen at the bottoms of the tubes because of the sedimentation of bacterial clumps. In milks highly contaminated with bacteria, TTC and NT seem to be equal with methylene blue in the rapidity of reduction, while the use of BT is hampered by its slower reduction.

As is known, room temperature (19° to 21°C) is inapplicable to the methylene blue test because reduction occurs in a reasonable time only with milks highly contaminated with bacteria. In contrast, it is a definite advantage of tetrazolium salts that they may be employed at room temperature when milk samples with high bacterial contents are screened under field conditions. The formation of formazan is relatively little delayed in such milks by room temperature.

When the effect of light on the reduction of the tetrazolium salts was studied, it was found that TTC was the most photosensitive. Even plain water solutions of TTC became red under the influence of light, whereas the water solutions of NT and BT were stable. In milk the reduction of tetrazolium salts is accelerated by the light more than the reduction of methylene blue. The reduction in light was partly nonenzymatic, for boiled samples of milk also exhibited formazan formation on the side of the tube directed toward the light source. The higher the concentration of tetrazolium salts in the milk, the more photosensitive the reaction and the poorer the correlation between the reduction time and the bacterial content. We can agree with Schönberg (*2*) that the tetrazolium-reduction test performed in a diffuse daylight of unknown intensity using TTC in a final concentration of 10^{-3} yields inconsistent results concerning the bacterial content. Therefore, the tests should be conducted in complete darkness.

Thirty-eight percent formaldehyde up to a final concentration of 5×10^{-3} accelerated the reduction of methylene blue, but this concentration was definitely inhibitory to the formation of the formazans. Aldehydeoxidase of Schardinger seems, therefore, to play a smaller role in the reduction of tetrazolium salts than it does in the reduction of methylene blue. Salicylic acid was found to inhibit equally the reduction of the tetrazolium salts and that of methylene blue.

When a continuous stream of air was conducted simultaneously into identical tests with methylene blue and with the tetrazolium salts, it was found that methylene blue was not completely reduced to the leuco form, whereas the reduction of the tetrazolium salts was only slightly delayed. The stability of formazans against oxygen seems to be a great advantage of the tetrazolium salts over methylene blue, which is readily reoxidized by the atmospheric oxygen (*4*). Moreover, the number of bacteria that are capable of reducing tetrazolium salts can be estimated by microscope since the intensely colored microcrystals of formazan are intracellularly deposited in the bacteria.

Our attempts to develop a quantitative colorimetric method by extracting the formazans from milk with lipid solvents (*5*) were unsuccessful. Quantitative yields were not recovered with the use of ether, chloroform, benzene, toluene, xylene, methanol, ethanol, propanol, butanol, amyl alcohol, and their combinations with glacial acetic acid.

Table 1. Reduction times of the tetrazolium salts and methylene blue (MB) as influenced by the bacterial content of milk samples

Bacteria (No./ml)	Reduction time (min)			
	TTC	NT	BT	MB
$< 10^5$	$>$ 420	$>$ 420	$>$ 420	$>$ 660
10^5 to 5×10^5	420 to 270	420 to 270	420 to 270	660 to 360
5×10^5 to 4×10^6	270 to 90	270 to 90	270 to 90	360 to 120
4×10^6 to 20×10^6	90 to 25	90 to 15	90 to 60	120 to 20
$> 20 \times 10^6$	$<$ 25	$<$ 15	$<$ 60	$<$ 20

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5 July 1955

Fluorescence Characteristics of 5-Hydroxytryptamine (Serotonin)

Although no definite function has been ascertained for 5-hydroxytryptamine (5-HT), it is already evident from the reports of numerous investigators that it may be an important physiological agent. The most interesting development is the possible connection between 5-HT and brain function as first suggested by Gaddum (*1*), and indicated by recent studies of Shore, Silver, and Brodie (*2*).

To obtain more information about this compound, it is necessary to develop methods for its analysis in tissue extracts. A number of bioassays for 5-HT have been reported (*3, 4*) and these have contributed important information concerning the distribution of this compound. However, standard chemical procedures are not sufficiently sensitive to detect the small amounts that are present in tissues such as brain and blood.

In a previous communication, it was reported that 5-HT in dilute acid or at neutral *p*H fluoresces at 330 mμ when activated at 295 mμ (*5*). The fluorescence in the ultraviolet was detected with a specially designed spectrophotofluorometer. The sensitivity of the fluorescence in the ultraviolet is such that less than 0.1 μg can be readily measured. However, extracts of certain tissues such as brain contain other materials that fluoresce near 330 mμ and therefore make analysis difficult.

It has now been found that in stronger acid (3*N* HCl), 5-HT and other 5-hydroxyindoles fluoresce at 550 mμ and

that this fluorescence is also maximally activated at 295 mμ. This shift of fluorescence from the ultraviolet to the visible, with increasing acidity, is completely reversible and is not accompanied by a noticeable change in the absorption spectrum. No corresponding shift in fluorescence is observed with tryptamine, which is activated at 275 mμ and fluoresces at 360 mμ. In fact, tryptamine and tryptophan fluorescence disappear in 3*N* HCl. Obviously, this shift in fluorescence of 5-HT in strong acid must be related to the phenolic group.

Although the significance of this phenomenon is not understood, it can be useful as a method for assaying 5-HT. The visible fluorescence is almost as intense as that at 330 mμ and offers as an additional advantage the possibility of using available fluorescence measuring devices. Where instruments are available for measuring both peaks of fluorescence, the ability to shift the fluorescence from 330 mμ to 550 mμ and back offers additional specificity, since other indoles such as tryptophan do not do this. The factor of specificity is of great importance in measurements on tissue extracts.

A specific method for the determination of 5-HT in brain, based on measurement of 550 mμ fluorescence, has already been developed (*6*).

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11 July 1955

Two Carbohydrases Occurring in Insect-Produced Honeydew

By fractionally precipitating a concentrated aqueous solution of honeydew with ethanol, we have obtained fractions free of sugars that possess definite carbohydrase activities. We have obtained these enzymes from honeydews of the cottony-cushion scale, *Icerya purchasi* Mask. and the soft scale, *Coccus hesperidum* L. These honeydews are different types; the former have melezitose [α-D-G_p-(1→2)β-D-Fru_f-(3←1)-α-D-G_p] and the latter have gluco-sucrose [α-D-G_p-(1→4)-α-D-G_p-(1→2)β-D-Fru_f] as their principal trisaccharides.

Of various carbohydrates containing α- and β-glucosidic, β-fructosidic, and α-galactosidic linkages, only free sucrose would serve as a substrate. In both cases, the enzymes react with sucrose to produce a trisaccharide and monosaccharides. Cottony-cushion enzyme produces melezitose, glucose, and fructose; soft-scale enzyme produces gluco-sucrose, glucose, and fructose. In both cases, the appearance of free glucose must require isomerase activity, for more than half of the monosaccharides produced is glucose. Whether this is a function of a separate enzyme or not is not apparent at this time; suffice it to say that there is no reaction discernible when glucose and fructose are used as a substrate. Both enzymes have little or no hydrolytic activity on either of the trisaccharides they produce even though they both appear to act as α-glucosylases that require hydrolytic action as the initiating step for their synthesis reactions.

The evidence at hand indicates that these enzymes are produced by the insects rather than that they are present in the sap of the host plant. Transcarbohydrases have been reported by Giri *et al.* [*Science* **121**, 898 (1955)] from rat liver but this is, to our knowledge, the first instance of this type of enzyme from insects.

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11 July 1955

Graphic Determination of Mean Values

In a recent communication, G. W. Hervey (*1*) commented on a graphic method for determining the mean value of three ordinates presented in a paper on moving averages (*2*). Earlier references have been found (*3*); an essentially equivalent procedure, the location of the centroid of a triangle, dates back to the writings of Archimedes (*4*).

However, the extension of this type of technique to apply to any number of points was described only recently (*5*), and further investigation (*6*) of the properties of centroids has led to other graphic methods. The center of gravity of the points charted on a scatter diagram represents simultaneously the mean values of both the x and y variables. If the number of points can be factored into powers of 2 and 3 only, the graphic location of the centroid is straightforward and rapid. For example, with 24 ($2 \times 2 \times 2 \times 3$) points, joining neighboring points in pairs and marking the midpoints of the 12 segments so obtained will lead to 12 points, each representing two of the original 24. Repeating this process will reduce the number of points from 12 to 6, and then from 6 to 3. Finally, the centroid of these 3 points may be found by joining them to form a triangle, marking the midpoints of the sides, and drawing the medians from each angle to the midpoint of the opposite side. The common intersection point of these three medians represents the centroid of the original 24 points. Standard deviations (*7*) as well as regression lines and correlation coefficients (*8*) may then be calculated by various geometrical methods related to "crab addition" (*9*). Adaptations of the technique to cases in which the number of points contains factors other than 2 and 3 are being completed.

A study of centroids of unequally weighted points has resulted in the development of a number of convenient graphic procedures for handling numerical data. These include graphic methods for determining mean rate of change (*10*), fitting of straight lines according to the criterion of least squares, approximate integration by Simpson's rule, estimation of moments of area, and calculation of mean values and standard deviations for frequency distribution diagrams. Papers describing these techniques are in preparation.

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7 July 1955