been used intentionally in referring to the authors of the individual reviews. Each is an active contributor to the literature of his own particular field; it is unusual to find contributions of a critical character by such investigators as Kay, Pauli, Hans Fischer, Bloor, Waldschmidt-Leitz, Gortner, the Coris and Collip in a single volume.

The attempt to include within the compass of 550 pages the literature of the vast field of biochemistry has resulted in the omission of the discussion of many important papers. Harris, who writes concerning vitamins, although citing more than three hundred references to recent investigations, well presents the point of view which authors of reviews of this sort must of necessity accept. "The space allotted has enabled us to deal with no more than about one quarter of the total number of papers published during the year. It deserves to be said that of the large number thus crowded out the great majority represent some definite addition to knowledge, filling in some detail or other on the big canvas. We make this point because superficial critics so often suggest that out of this immense annual output of papers only comparatively few can be of real permanent value. This criticism seems to the reviewer to overlook the essential fact that (as all past experience goes to prove) progress is made only by the cumulative and cooperative efforts of many different workers, each adding his contribution to the general flow of knowledge. Solitary isolated advances are few. And much work is nevertheless useful and essential, although it represents no fresh development of theory, and finds no place in our review. . . ."

The editors believe that "even at the expense of omitting references to many papers,—critical surveys of the literature, though less comprehensive, are of more value to users of the Review than uncritical compendia." This ideal of a critical survey has been maintained with few exceptions. Unfortunately, one of these, which is little more than a catalogue of abstracts and references, is concerned with one of the most important subjects in biochemistry.

Space does not permit discussion of the individual reviews. The surveys of the difficult fields of hormones and vitamins are of the same excellent workmanship which has characterized the previous reviews by these authors in earlier volumes. Particular reference may be made to the valuable résumé of the important subject of energy metabolism in the review of nutrition by Professor Brody, an author who has not previously contributed to this work. The criticism, which has been made frequently, that biochemistry in America is considered chiefly in its relations to the animal organism and to medicine, finds no justification in the present volume. Plant chemistry and nutrition are amply covered by the discussion of the terpenes and saponines, the nitrogenous constituents of green plants, mineral nutrition, metabolism of carbohydrates and organic acids in plants and the chemistry of bacteria.

Professor Luck and his collaborators have again rendered valuable service to biochemistry and related sciences. The review well deserves the support of all those interested in these fields.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

RAPID STAINING METHODS

NEGATIVE STAINING OF MICROORGANISMS

THE so-called negative staining is frequently very useful in studying bacteria, yeasts and other microorganisms. To make mounts the organisms are transferred to a small drop of dye on a slide, spread in a thin layer and allowed to dry. They will then appear as colorless objects in a stained background. The dyes commonly used for this purpose are aqueous solutions of nigrosin (1 per cent.) or Congo red (2 per cent.). After the film of Congo red has dried, the color may be changed to blue by adding a drop of 1 per cent. hydrochloric acid in 95 per cent. alcohol. For examining such preparations either immersion oil or Nujol should be placed directly on the film.

In making negative preparations the writer has found that the addition of certain other dyes to aqueous nigrosin is very advantageous. The use of such mixtures results in various colors of background and, in some cases, in light staining of the organisms. Many dyes have been tried, but the most satisfactory ones are rose bengal, Magdala red, cotton blue and acid fuchsin. A 1 per cent. aqueous solution of any one of these dyes, excepting acid fuchsin, is added to a 1 per cent. nigrosin solution in the proportion of 1 to 3. Traces of acid may be added to the nigrosin-cotton blue mixture, but should not be added to the other two mixtures (nigrosin and rose bengal or Magdala red), although the addition of a little alkali to them may be advantageous. Of course the proportions of the dyes in the mixtures may be varied as desired. In the case of acid fuchsin 30 drops of the dye solution (1 per cent.) and 4 drops of concentrated hydrochloric acid are added to 40 cubic centimeters of aqueous nigrosin.

After films of nigrosin alone, or in combination with another dye, have dried they may be fixed to the slide, so that they will not wash off readily with water by covering with a drop of 2 per cent. ferric chloride and, after a minute, washing with water.

The nigrosin-cotton blue combination is very good as a background for stained mounts (Dorner's method or modifications of it) of spores of bacteria or yeasts, vegetative, capsulated or slime-producing bacteria, small asci and spores of fungi (Gibberella, Diaporthe, Dothidella, etc.), some protozoa and other small organisms. The arrangement of the cilia of Paramoecium in such mounts can be seen well enough to count them readily.

Generally there is little difference in the results obtained with nigrosin combined with rose bengal or with Magdala red. They have been used satisfactorily for vegetative, capsulated or spore-bearing bacteria, vegetative yeasts, spores and asci of fungi, and germinating spores of smuts to show promycelia and sporidia. If one introduces the material to be examined into the dye mixture and allows the preparation to dry the cells will be colorless, or nearly so, in a stained background; but if the stain is added to a *dry* smear (bacteria, yeast, germinating smut spores) and allowed to dry the vegetative cells will stain a shade of pink or red. This makes it possible to demonstrate clearly spores of bacteria, especially with nigrosin plus Magdala red, the preparations showing colorless spores in pink cells.

Nigrosin combined with acid fuchsin is exceptionally good for demonstration of spores in bacteria and yeasts. Spores of bacteria will appear much the same as described for Magdala red; but with yeast, if the cultures are used soon after they begin to form spores, the mother cell or ascus appears colorless, while the spores stain pink. Smears of either bacteria or yeast should be allowed to dry on the slide before the dye is applied.

A Rapid Staining Method for Dividing Cells

Although there are numerous methods of staining dividing cells to show mitotic figures, most of them are rather time-consuming. The following procedure requires a minimum of time and results in excellent preparations which are exceptionally clear and transparent. The method has been used especially for cell division in root tips and in anthers of *Lilium,* following fixation with chromo-acetic or Flemming's fluid. It is easier than triple staining and well adapted for the beginner. All reagents may be kept in pipette bottles.

After removing the paraffine from sections treat them in order with absolute, 95, 70, 50 and 30 per cent. alcohols, followed by water. In doing this put 2 or 3 drops of each in succession on the sections, let each act 20 to 30 seconds and drain before adding the next one. Then stain on the slide for 3 to 5 minutes with anilin-alcohol-fuchsin.[1]

Water, distilled	30 cc
Basic fuchsin, 10 per cent. alcoholic	10 "
Anilin oil (1 part) and 95 per cent. alcohol (3 parts) mixed	5 "
Acetic acid, 4 per cent.	1 "

Mix in the order given, filter once or twice and again before using. This solution is best after standing three to six days and generally works well for three to five weeks. Refiltering may be necessary.

Wash with 3 or 4 changes of water and then dehydrate with the alcohols to 95 per cent. alcohol containing a trace of hydrochloric acid (for destaining), then with 95 per cent. and absolute alcohol. Counter stain with one tenth per cent. solutions of Orange G and light green in clove oil (mixed in proportion of 1:3), wash off with absolute alcohol, clear with xylol and mount in balsam. Anilin blue in 90 per cent. alcohol is also a very good counterstain.

This entire procedure will require about eight minutes and, after a little practise, one can stain seven or eight slides per hour. When staining jars are used for the different grades of alcohol, they soon become colored with dye. In the method outlined the alcohols, stains and xylol are discarded after being used once. However, this is not wasteful as the amount of material thrown away in making a slide is not more than two or three cubic centimeters, and one has the satisfaction of always working with clean chemicals.

Light green in clove oil gives a beautiful contrast, but is certain to fade after some time. Orange G alone may overstain quickly, but when mixed with light green it stains less deeply and one gets a greenish shade that will be visible for some months, after which the Orange G alone remains.

Very fine preparations may also be made by substituting crystal violet for basic fuchsin in the formula given above. Or one may obtain a red tinged with violet in the chromatin by staining with the crystal violet three to five minutes and, after washing with water, staining three to five minutes with the basic fuchsin.

Another variation is to use safranin instead of basic fuchsin, made according to the following formula:

Safranin, 3 per cent. in absolute alcohol	20 cc
Distilled water	20 "
Alcohol, 95 per cent. (3 parts) plus anilin oil (1 part)	5 "
Counterstain with anilin blue in 90 per cent. alcohol.	

[1] W. E. Maneval, "Some Staining Methods for Bacteria and Yeasts," *Stain Technology,* 4: 21–25, 1929.

The anilin-alcohol-safranin is also very satisfactory for staining sections of stems and leaves. The stain may be prepared as above with the addition of one cc of 4 per cent. acetic acid. After the usual procedure, stain 3 to 10 minutes, wash with water, counterstain with ½ per cent. cotton blue in 70 per cent. alcohol for a few seconds, dehydrate, clear and mount in balsam. The entire process is carried out on the slide, staining jars being unnecessary.

Another combination of dyes that is apparently as good, or nearly as good, as safranin and cotton blue for stem and leaf sections is anilin-alcohol-basic fuchsin (3 minutes) followed by Delafield's haematoxylin.

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THE DETERMINATION OF CO_2 CONTENT OF AN ATMOSPHERE IN A CLOSED SYSTEM[1]

IN a previous publication[2] a colorimetric method was described for the determination of the CO_2 content of an atmosphere. This method was based on the estimation of pH in a standard $NaHCO_3$ solution in equilibrium with the CO_2 in the atmosphere. As described, the method was not applicable to closed systems; since many physiological studies make use of closed containers, two modifications have been devised which can be used in these studies.

Method 1: This is a modification of a method described by Osterhout for measuring the respiration of bacteria.[3] The atmosphere from the closed system is circulated through a 13 mm test-tube fitted with an inlet tube drawn to a 1 mm capillary tip and containing approximately 4 cc of $NaHCO_3$ solution plus indicator. The latter is prepared by mixing 1 cc of a given indicator with 20 cc of 0.001N $NaHCO_3$. The gas is circulated through the solution by means of an ordinary aspirator bulb and returned to the original container. About 20 aspirations of the bulb circulates enough gas to bring the solution to equilibrium with the atmosphere; its pH is determined by comparison with a standard Hellige color disk and the pCO_2 read from the standardization curve.[2] For cresol red or brom-thymol-blue the equation of this curve is:

$$\text{Log } pCO_2 = 7.30 - pH$$

This method is fairly accurate and more rapid than any heretofore suggested. However, if several determinations must be made at short intervals, a second method which automatically indicates the pCO_2 proves more satisfactory.

Method 2: An automatic determination of the pCO_2 in the atmosphere of a closed system can be obtained by suspending a 25 x 50 mm tube containing 2 cc of the $NaHCO_3$ plus indicator solution inside the closed system, with provision for addition and withdrawal of the solution. At any time the CO_2 content of the atmosphere can be determined from the color of the solution. For accurate work, the pH can be estimated by comparison with standard buffer solutions (2 cc in a 25 x 50 mm tube). With a little practise, however, the operator can judge the pH of the solution without the use of these standards. Although this device can be used to estimate the actual CO_2 content of the atmosphere in a closed system, it is of especial value for notifying the operator when the CO_2 has reached a given predetermined level. In the latter case, an indicator is used which exhibits a pronounced color change at a pH corresponding to the pCO_2 desired.

Tests of the methods: To test the methods, a known quantity of CO_2 was added to the atmosphere in a closed system. After 20 minutes, the time found to be necessary for the suspended solution to reach equilibrium with the atmosphere, the pCO_2 was estimated by the two methods; the buffer standards were used in the automatic method to insure greater accuracy. Both methods gave satisfactory results in tests of atmospheres whose CO_2 content ranged from 0.03 to 0.7 per cent. The estimations checked the actual quantity of CO_2 within 5 to 10 per cent., which is the limits of accuracy for the colorimetric method.

In connection with various physiological studies both methods have proved reliable in greenhouse experiments. The second method is particularly useful when CO_2 must be added periodically to a closed container. An indicator is selected which has a definite color change at a pH corresponding to the lowest level of CO_2 desired. For example, if it is required to keep the CO_2 level greater than 0.15 per cent., phenol red is an appropriate indicator, since at this concentration of CO_2 it turns from a definite yellow to a definite pink. If the CO_2 is not to be added until reduced to the concentration of air, cresol red is satisfactory. Greenhouse tests on the second method carried out over a period of six months show that the color change of the indicator corresponds to a quite definite pCO_2 in the atmosphere and that the method can be entirely relied upon to indicate when CO_2 shall be added to plants in physiological experiments. The indicator solution used should be changed at least every three days for highest accuracy.

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[1] Herman Frasch Foundation in Agricultural Chemistry, Paper No. 80. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

[2] P. W. Wilson, "Colorimetric Method for Determination of CO_2 in Gas Mixtures," SCIENCE, 78: 462–463, 1933.

[3] W. J. V. Osterhout, "A Method of Studying Respiration," *Jour. Gen. Physiol.*, 1: 17–22, 1918.