TABLE 1

| | Exposure to $N_2$ | Activity (counts/minute)[a] |
|---|---|---|
| Barley[b] ..... | 20 min. | 200 ± 4 |
| Freshly killed barley | " " | 2 ± 2 |

[a] At the time of counting.
[b] 30 grams fresh weight.

ity had the $N^{13}$ half-life. The plants assimilated $10^{-4}$ to $10^{-5}$ of the available $N_2$*. For the experiment shown in Table 1 this corresponds roughly to 0.01 cc of $N_2$. Although the experimental conditions are widely different and a quantitative comparison is difficult, it is of interest to note that this figure is of the same order of magnitude as the rate of $N_2$ fixation calculated from the data of Lipman and Taylor.[2]

These experiments with $N^{13}$ do not necessarily prove that a net uptake of $N_2$ has occurred, since the existence of reversible (interchange) reactions involving $N_2$ is possible. This possibility, however, seems rather remote, and therefore it is not unreasonable to consider these experiments as positive evidence for $N_2$ fixation by non-leguminous plants.

Due to the magnitude of the assimilation it was not possible to determine into what compound the $N_2$ was converted. However, experiments are in progress to study the mechanism of $N_2$ fixation by the known $N_2$ fixing organisms (Azotobacter, legumes, etc.). It is unfortunate that a longer-lived radioactive nitrogen isotope is not available. It is apparent, however, that stable $N^{15}$ can be used more effectively in a study of these problems.

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S. RUBEN
W. Z. HASSID
M. D. KAMEN

UNIVERSITY OF CALIFORNIA,
BERKELEY

# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## HEPARIN AS AN ANTICOAGULANT FOR PERMEABILITY STUDIES[1]

NUCLEATED erythrocytes, especially those of the chicken, have been used in this laboratory for studies of permeability and respiration. For measurements of oxygen consumption the nucleated cells are much more satisfactory than the enucleated erythrocytes of the mammals. This is due to the difference in rates of oxygen consumption between these two types of cells. For permeability studies, however, the mammalian cells are much easier to use. Nucleated erythrocytes in slightly acid media, for example, behave in an abnormal fashion. In attempting to investigate the effect of pH on osmotic hemolysis in chicken erythrocytes it was necessary to centrifuge the cells several times. When attempts were made to resuspend cells after this treatment it was often found that they had formed a sticky mass which could not be broken up. Other experiments in which partially hemolyzed cells were centrifuged gave similar results. In all of these experiments the blood had either been defibrinated or oxalate had been added to prevent clotting.

In experiments in which heparin was used as an anticoagulant (Glogau—1 mg per 10 cc of blood) it was found that the cells could be treated in the manner described above, with much less tendency for them to stick together in a stringy mass. It would seem, then, that heparinized chicken blood left the cells in a condition much more suitable for experimentation.

Circumstantial evidence would suggest that this difference in behavior might be due to differences in the $Ca^{++}$. In both defibrinated blood and oxalated blood the $Ca^{++}$ is below the normal level. In heparinized blood, however, the $Ca^{++}$ should be at the normal level. Although the direct effect of $Ca^{++}$ and its interaction with other ions such as $Na^{+}$ and $K^{+}$ are controversial subjects (for a general discussion of this problem see Heilbrunn[2]), there are a large number of data which indicate that protoplasmic properties such as viscosity, etc., are dependent on $Ca^{++}$. A detailed investigation would be necessary before one could be certain that a lowered $Ca^{++}$ caused the sticking together of these cells.

The authors recommend, then, the use of heparin as an anticoagulant in investigations in which nucleated erythrocytes are subjected to much experimentation. The cells appear to be more nearly normal, and have less tendency to stick together in an unusable mass.

F. R. HUNTER
L. D. STRINGER
H. D. WEISS

RHODE ISLAND STATE COLLEGE

## A NEW TECHNIC FOR STAINING VAGINAL SMEARS: II[1]

IN a recent communication to this journal[2] a new technic was described for staining vaginal smears, employing a modified Masson trichrome stain. This stain added a series of color changes to the cytological altera-

[1] One of the authors, F. R. Hunter, is indebted to the American Association for the Advancement of Science and to the American Academy of Arts and Sciences for grants-in-aid.

[2] L. V. Heilbrunn, "An Outline of General Physiology," W. B. Saunders Co., Philadelphia, 1937.

[1] Aided by a grant from the Josiah Macy, Jr., Foundation.

[2] SCIENCE, 91: 321, 1940.

tions in the smears, greatly facilitating their interpretation. Attempts to simplify the technic as well as to avoid the necessity for relying on imported stains such as Ponceau de Xylidene and Light Green have since been carried out. These have been greatly facilitated by the report of Lillie[3] that domestic Biebrich Scarlet and Fast Green FCF may be substituted for Ponceau de Xylidene and Light Green respectively; and that a mixture of equal parts of 5 per cent. phosphomolybdic and phosphotungstic acids gives adequate mordanting in one minute. On this basis, it has been possible to simplify and shorten the technic previously described for the vaginal smear and use domestic stains exclusively.

The revised staining technic embracing these modifications is as follows:

(1) From fixing solution, carry through alcohols to water; stain with Harris Hematoxylon for 2 minutes, and wash in running water for 5 minutes.

(2) Instead of the Ponceau de Xylidene-Acid Fuchsin-Orange G solution, 1 per cent. Biebrich Scarlet, water soluble (Nat'l Aniline and Chem. Co.) and 0.4 per cent. Orange G in 1 per cent. acetic acid. Stain 1 minute and rinse in water.

(3) In place of the 3 per cent. phosphotungstic acid mordant, a mixture of equal parts of 5 per cent. phosphomolybdic and phosphotungstic acids. Mordant 1 minute and rinse.

(4) In place of 0.3 per cent. Light Green, a 0.25 per cent. solution of Fast Green FCF (Nat'l Aniline and Chem. Co.) in 0.3 per cent. acetic acid. Stain 2 minutes. Do not rinse.

(5) Differentiate in 1 per cent. acetic acid for 1 minute, carry through alcohols to xylol and mount in damar.

It is possible to omit the hematoxylin stain under certain conditions, as in the routine treatment of the menopause with estrogens. With this omission, the smear can be stained in 5 minutes.

The assistance of Eugene J. Cohen in working out these modifications is gratefully acknowledged.

EPHRAIM SHORR

THE NEW YORK HOSPITAL AND
THE DEPARTMENT OF MEDICINE,
CORNELL UNIVERSITY MEDICAL
COLLEGE, NEW YORK, N. Y.

## SOLUTIONS OF CHLOROPHYLL IN SALT WATER

ALTHOUGH a number of workers have studied aqueous extracts of chlorophyll from fresh leaves, only Inman[1] seems to have discovered that the addition of salt to the water is beneficial. Since Inman seems never to have published his findings in this respect, and since the author hasn't time to do adequate research with the method, it seems worth publishing this statement.

As various workers have stated, chlorophyll can be suspended in water if fresh leaves are ground in water, either with or without an abrasive. However, the suspended chlorophyll settles out within a few hours (with a few exceptions). Smith[2] has found that the addition to the colloid solution of a detergent will keep the chlorophyll in suspension. Less drastic treatment than that will stabilize the colloid. It is only necessary to grind the leaves with a salt and water solution rather than pure water.

$Na_2SO_4$ and NaCl have been found effective. The optimum concentration for NaCl is between 2 per cent. and 5 per cent. Since it has seemed desirable to control the pH, M/15 phosphate buffer of pH 7 is being used at present, and it gives very satisfactory solutions. $CaCl_2$ will not maintain the colloid in suspension. Buffers of pH 6 and below are not satisfactory, for the chlorophyll tends to decompose. Borate buffers at pH's 8 and 11 seem satisfactory, but it is feared that the high pH may change the chlorophyll in some way.

The chlorophyll suspension obtained in salt solutions is never clear. It possesses the various properties reported heretofore. It is relatively photostable, is precipitated by protein coagulants, passes through filter paper, is difficult to centrifuge down, has the red absorption band in the same place as that of an intact leaf, behaves as if negatively charged in cataphoresis, can be precipitated by ammonium sulfate and redissolved by addition of fresh buffer solution.

JOHN SHAFER, JR.

CORNELL UNIVERSITY

[3] *Stain Technology*, 15: 17, 1940.

[1] O. L. Inman and M. L. Crowell, *Plant Physiol.*, 14: 388–390, 1939; also in private conversation.

[2] E. L. Smith, SCIENCE, 91: 199–200, 1940.

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