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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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.

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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.