fixed in 10 per cent. formalin and stained by Gömöri's[4] method for the demonstration of reticulum and collagen. This procedure stains reticulum black and collagen old-rose.

These studies support the view that reticulum is a precollagenous type of connective tissue and may be transformed into collagen. A transitional phase was found between the two. The uterine connective tissue of the 15-day rat was chiefly reticulum. In the endometrium a fine reticular network extended from beneath the lining epithelium, where it was condensed to form a basement membrane, to or almost to the circular smooth muscle (C.S.M.). A narrow zone of collagen usually bordered the C.S.M. A network of reticulum was found about the muscle cells in both the circular and longitudinal smooth muscle (L.S.M.) layers. Peripheral to the L.S.M. and underlying the serosa a condensation of narrow collagen fibers formed a subserosal layer. Numerous trabeculae extended inward and divided the L.S.M. layer into irregular columns of cells. The connective tissue of the vaginal mucosa was definitely collagenous, although immediately below the epithelium a narrow line of argyrophilic material was found.

In increasing older rats there was in the uterus a gradual transformation of reticulum into collagen and a definite increase in the amounts of collagen which was most marked in the very old animals. Such changes occurred earliest and most markedly in the endometrium. In rats over six months of age collagen extended from the C.S.M. practically to the lining epithelium, although a reticular basement membrane almost invariably persisted even in the oldest rats. Here there was a gradual thickening of the collagen fiber bundles which often measured as much as 12 micra. As age increased there was a tendency for the reticulum of the C.S.M. and the L.S.M. to be transformed into collagen. These changes, however, were extensive only in rats over eighteen months of age. The subserosal layer and the trabeculae penetrating the L.S.M. became markedly thickened. Throughout life, and especially in the very old animals, there was an increase in the size and density of the collagenous fibers of the vaginal mucosa.

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RADIOACTIVE NITROGEN IN THE STUDY OF N_2 FIXATION BY NON-LEGUMINOUS PLANTS

THERE has been considerable controversy regarding the ability of non-leguminous plants to fix atmospheric nitrogen. Lipman and Taylor[1,2] and many others have presented positive evidence for N_2 fixation by wheat, barley, etc. In the experiments of Lipman and Taylor plants were grown both in the presence and absence of nitrogenous salts and were analyzed by a modified Kjeldahl method. The results showed that the plants contained more N than could have been obtained from the growth media. However, their conclusions have not been generally accepted.

It should be possible, using radioactive nitrogen, to obtain additional information regarding this problem. Despite the 10.5 minute half-life of N^{13} the yields obtainable in the Berkeley cyclotron are such as to make this feasible. The nuclear reactions are:

$$ {}_1D^2 + {}_6C^{12} \rightarrow {}_7N^{13} + {}_0n^1 \text{ and } {}_7N^{13} \rightarrow e^+ + {}_6C^{13}. $$

Carbon (charcoal) was bombarded in a gas-tight chamber incorporating many features found to be of value in the Radiation Laboratory for the handling of high intensity beams. The active gas in the target holder was passed through a heated combustion tube containing cupric oxide into a pyrex desiccator containing the barley plants. Only the tops were used; the plants were cut well above the roots to eliminate any bacteria clinging to the roots. The charcoal which contained the major fraction of the N^{13} was introduced into the combustion tube and burned in a stream of O_2. As a control an equal weight of barley killed by immersion in boiling water was present in the desiccator.

After the plants were exposed to the N_2^* for ~ 20 minutes they were removed and extracted with boiling 80 per cent. ethanol. After filtration the extract was boiled vigorously in a stream of air. The method of counting has been described elsewhere.[3] In the first experiments both the live and dead plants were found to contain N^*. This was due to the presence of radioactive combined nitrogen (*i.e.*, CN, NH_3, NO, etc.) produced by the recoiling N^{13} atoms during the bombardment with the energetic (8 MEV) deuterons. The N_2^* was purified of combined nitrogen by slowly passing the active gases over heated CuO and then through a series of five traps and spirals immersed in liquid air. Before entering the trap system the gas stream was passed through solutions of HNO_3 and NH_4OH. The effectiveness of the purification was shown by the fact that no traces of NH_3 could be detected with Nessler's reagent, which is extremely sensitive for small amounts of NH_3.

Observing these precautions the live plants were found to contain N^*, while no activity (< 1 per cent.) could be detected in the control plants. The results of a typical experiment are shown in Table 1. The activ-

[4] G. Gömöri, *Am. Jour. Path.*, 13: 993–1001, 1937.

[1] Lipman and Taylor, SCIENCE, 56: 605, 1922.
[2] Lipman and Taylor, *Jour. Frank. Inst.*, 198: 475, 1924.
[3] Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, 61: 661, 1939.

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.

We are indebted to Professor E. O. Lawrence and members of the Radiation Laboratory for their interest and cooperation.

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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.