The results of the laboratory studies relating to the fate of the nitrogen fixed in the soil are represented in Fig. 1.

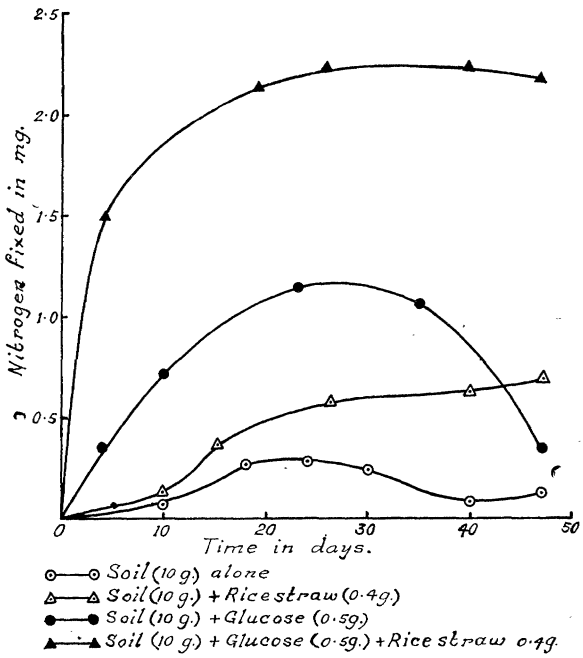

FIG. 1

These results show that the fixation of nitrogen under these conditions is almost immediately followed by its loss, so that both the processes proceed simultaneously till about three weeks, when the period of maximum fixation is reached, and during this period the rate of fixation is obviously greater. The loss continues steadily even after this period so that, within the following three to four weeks, more than 60 per cent. of the fixed nitrogen disappears from the soil system. This, combined with the fact that a period of rest extending up to four weeks is necessary between fertilizer application and sowing or transplanting, would indeed show that the crop can not fully utilize the nitrogen fixed by these materials. In presence of straw in the medium, however, the loss is prevented and the fixed nitrogen is retained in the system for a longer time.

The same type of loss is also observed when nitrogen in the form of dried Azotobacter cells is added to the soil (Table 2).

TABLE 2

Time in days:	5	7	12	17
Nitrogen lost in mg from 10 g of soil	0.74	0.80	1.08	1.62

It has also been observed that this loss of biologically fixed nitrogen can be prevented to an appreciable extent by the addition of cellulosic materials like straw.

Probably this loss of fixed nitrogen is of a general character in the soil even under natural conditions, more especially in tropical climes; and this same loss which is going on to a lesser extent to which natural fixation is also taking place, may have been magnified under the above experimental conditions. Full details of the experiments will be published elsewhere.

Experiments with a view to finding out the exact mechanism of this loss and methods of preventing it in actual field operations are in progress.

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THE AVAILABILITY OF THE IRON IN DRIED PEAS AND BEANS

SINCE dried peas and beans have been considered important sources of dietary iron on the basis of their relatively high content of this element, an investigation has been made of the actual availability of the iron in blackeyed peas, green and yellow split peas, navy beans, pinto beans and butter beans. Blackeyed peas, which had been cooked preliminary to drying and grinding, were found to have all the iron available.[1] The details of the biological technic used may be found in a previous report upon the availability of iron.[1] In general the method consists of measuring the rise of hemoglobin in rats rendered anemic by an exclusive milk diet in response to the addition of the test food.

The process used in preparing the dried peas and beans for feeding consisted of drying them at 60° to 65° C., breaking in a mortar and grinding in a ball mill until the particles were fine enough to pass through a 40-mesh sieve. Analyses of the dried products for total iron by Farrar's method showed the following values expressed in mg per gm: blackeyed peas, 0.087; green split peas, 0.053; yellow split peas, 0.053; navy beans, 0.077; pinto beans, 0.077; butter beans, 0.78.

The experimental animals were weaned at 21 days of age and placed on a diet of fresh whole milk. When the level of hemoglobin had fallen below 3.0 gm per 100 cc of blood, the test foods were added to the diet at levels providing 0.2 mg of iron per day throughout the 6-week experimental period. Positive controls were fed 0.2 mg of iron, as ferric chloride. Negative controls were kept on the milk diet alone as a check on its freedom from contamination. Each animal receiving an iron-containing supplement was given daily 0.05 mg of copper, as copper sulfate, to insure that hemoglobin synthesis might be as complete as possible.

[1] Ascham L. M. Speirs and D. Maddox, *Jour. Nutrition*, 16: 425, 1938.

The animals were distributed to the various supplements as evenly as possible in respect to age, weight and litter mates.

The gains in hemoglobin during the experimental period in response to the various food supplements are shown in Table 1. As is evident from the figures for the standard deviation of the mean (S. D.), small differences may not be considered significant for these values. The hemoglobin of the animals receiving the test foods and of those receiving completely available iron, in the form of ferric chloride, rose to approximately the same levels. It appears, therefore, that the iron in the dried foods tested, namely, blackeyed peas, green and yellow split peas, navy beans, pinto beans and butter beans, is completely available to the nutritionally anemic rat for the regeneration of hemoglobin.

TABLE 1

THE REGENERATION OF HEMOGLOBIN IN ANEMIC RATS RECEIVING EITHER FERRIC CHLORIDE OR FOOD SUPPLEMENTS CONTAINING 0.2 MG OF IRON PER DAY DURING AN EXPERIMENTAL PERIOD OF 6 WEEKS

Supplement	Number of rats	Gain in hemoglobin	± S. D.*
		gm/100 cc	
$FeCl_3$	25	9.3	0.26
Blackeyed peas	8	8.9	0.33
Navy beans	8	8.8	0.46
Pinto beans	9	8.9	0.42
Butter beans	9	9.3	0.31
Green split peas	7	10.1	0.54
Yellow split peas	6	10.3	0.20

* Standard deviation of the mean.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CULTURE TECHNIC FOR QUANTITATIVE GROWTH STUDIES WITH MYXOPHYCEAE

THE procedure whereby aliquots are taken for cell counts from liquid or other large volume culture media is inaccurate because of unequal distribution. And the hanging-drop method of slide culture is difficult to manage for long periods when frequent changes of solution must be had. Therefore when studies of cell increase in number are to be made of small organisms somewhat larger than bacteria, other methods must be devised. Two such methods are here reported.

Chroococcus was grown in tiny paraffin boxes fixed to glass slides. The boxes were made from the sprocket holes of 16 mm uncoated movie film kindly donated by the Eastman Kodak Company of Rochester, New York. Short strips of the film were washed overnight in distilled water, dried, roughened by fine sandpaper and coated with hard (62°) paraffin. Pieces containing a single rectangular hole were affixed to cover-slips by warming. Into the little receptacle so produced the blue-green algae cells were transferred by micropipette from a Bristol solution culture. Accurate counts were made of the cells so introduced at the beginning and after any desired period up to 72 hours. Evaporation was controlled by keeping the preparation in large petri dishes containing wet filter paper and by adding distilled water when necessary.

The second method was found useful for microorganisms which have a gelatinous sheath, such as Anabaena and diatoms. Thoroughly clean cover-slips were broken into tiny pieces, washed and placed in small petri dishes in such manner that each little piece of glass remained free from the others. The preparation was sterilized while wet so that the glass fragments became attached and but one surface was available for algae growth. Over this preparation was then poured about 10 cc of a Bristol solution culture of the organism and the whole was allowed to stand for from 24 to 48 hours. By that time algae were attached and growing on the fragments. Single pieces were then transferred to hollow ground culture slides, one to each, and the number of cells counted. Anabaena was kept healthy and increasing in cell number under such conditions for as long as 120 hours with many changes of solution, while the accompanying growth was quantitatively determinable at will.

Although most of the blue-green algae grow nicely on agar and such colonies serve adequately as stock material, it is next to impossible to obtain a quantitative sample therefrom. Consequently, the procedure of first transferring from agar to Bristol solution was adopted. Cell increase in this medium takes place readily, and clumps can be so broken by mild shaking that samples for quantitative study can be obtained as described above.

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AN INEXPENSIVE SMALL AIR COMPRESSOR

HOAGLAND[1] and others have stressed the desirability of adequate aeration of solutions if reliable experimental data are to be obtained from plants grown in culture solutions. Those working in greenhouses far removed from a source of compressed air are forced to choose between several rather unsatisfactory procedures. Small rotary pumps may not give sufficient pressure to assure air for all cultures. Conventional

[1] D. R. Hoagland, *Bot. Rev.*, 3: 307–334, 1937.