of these acids alone. This combined effect is illustrated particularly clearly by Hallman's[4] work with the heart muscle.

Detailed investigations have now convincingly shown that pyruvic acid and the 4-carbon acids act in the animal organism as primary sources of the enzymic citric acid synthesis. In these investigations it was possible, under suitable conditions, to produce in the heart muscle enzymically about 0.2 per cent. of citric acid within a short period of time. The most pronounced effect was noted when pyruvic acid together with malic acid was used as substrate under aerobic conditions. Table 1 shows the results of one of such combination experiments.

TABLE 1

20 g MINCED HEART MUSCLE; 50 ml BICARBONATE BUFFER. INCUBATED AEROBICALLY; 30 MINS. AT 37°.

Substances added	Na pyruvate 15.0 mg	Na malate 39.0 mg	Na pyruvate 15.0 mg Na malate 39.0 mg
Citric acid produced	1.2 mg	3.1 mg	25.0 mg

When larger quantities of malic and pyruvic acid were employed, under the above conditions, the amount of citric acid could be substantially increased. For instance, in an experiment with 325 mg Na pyruvate and 510 mg Na malate, 20 g heart muscle produced in 30 minutes 151 mg citric acid.

In the synthesis of citric acid, malic acid is obviously first dehydrated enzymically to oxalacetic acid, which then immediately reacts with the excess pyruvic acid to form an intermediate compound of citric acid. (That citric acid can be synthesized by purely chemical methods from pyruvic acid and oxalacetic acid, has been shown earlier by Knoop and Martius.[5])

To our surprise we found, however, that the effect of oxalacetic acid in the heart muscle was distinctly less pronounced than that of malic acid. The effect of fumaric acid was approximately equal to that of oxalacetic acid. In the other tissues examined, oxalacetic acid produced the best effects.

When boiled tissue was used, synthesis of citric acid could not be demonstrated—even in the presence of oxalacetic acid. Hence it can be concluded that under the conditions of our experiments, the citric acid formation is ascribable to enzymic processes.

It should also be observed that in the combination experiments the effect of *phospho-pyruvic* acid was definitely less than that of pyruvic acid alone.

Our experiments indicate that at least in the heart muscle—which, according to unpublished work of Hallman, effects a very powerful decomposition of citric acid—pyruvic acid is transformed to a large extent via citric acid. From the citric acid stage the decomposition follows the course discovered by Martius and Knoop,[6] to α-keto-glutaric acid and then further to succinic, fumaric, malic and oxalacetic acid. Following the union of the 4-carbon skeleton with pyruvic acid, to form the primary stage of citric acid, the cycle proceeds further. (It is probable that the vitamin B_1 plays an important role in the decarboxylation of this precursor of citric acid, formed from pyruvic and oxalacetic acids.) As a result of successive dehydrations and additions of water molecules, pyruvic acid is in this process ultimately burned to carbon dioxide and water, whereby each molecule of pyruvic acid requires 5 atoms of oxygen and produces 3 molecules of carbon dioxide.

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[4] N. Hallman, *Suomen Kemistilehti* (*Acta chemica fennica*) B, 12: 11, 1939.

[5] F. Knoop and C. Martius, *Zeits. f. Physiol. Chem.*, 242: I, 1936.

LOSS OF BIOLOGICALLY FIXED NITROGEN FROM SOILS AND ITS BEARING ON CROP PRODUCTION

IT is well known that application of readily fermentable carbohydrates to soil leads to increased microbial activity, resulting in the fixation of considerable quantities of atmospheric nitrogen. With a view to finding out how far the nitrogen thus fixed is available for plant nutrition, a number of pot and field trials were carried out with different crops. Using molasses (a waste product obtained from the local sugar factory which is rich in sugars, having a total percentage of from 50 to 60 per cent. of sugars), it was found that although useful amounts of nitrogen are fixed in the soil, the major part of it was somehow not available for plant growth. Thus, in a typical field experiment using Ragi (*Eleusine coracana*) the results given in Table 1 were obtained:

TABLE 1

Treatment	Yield of grain in gms.
Control (untreated)	3,560
Hongay cake	5,704
Sugars* (as molasses)	4,810

* The sugar (as molasses) was applied in quantities such that it fixes the same amount of nitrogen in the soil as supplied in the form of Hongay cake.

From the foregoing table, it is evident that only a part of the fixed nitrogen is rendered available to the crop; probably the rest is being lost from the soil system. Mirchandani[1] has reported a similar type of nitrogen loss.

[6] C. Martius and F. Knoop, *Zeits. f. physiol. Chem.*, 246: I, 1937.

[1] T. J. Mirchandani, *Proc. Nat. Inst. Sci.*, Vol. III, 185, 1937.

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

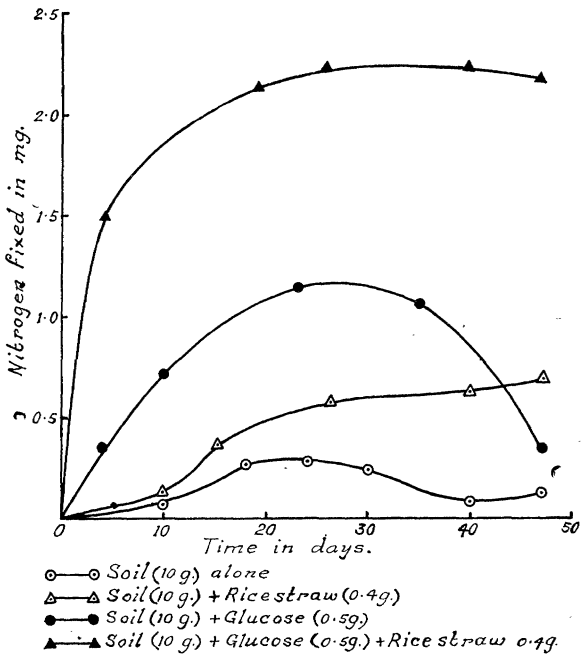

FIG. 1

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.