CXC. THE EXUDATION OF GLUTAMINE FROM PERENNIAL RYE-GRASS.

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(*Received June 25th, 1934.*)

During the course of an investigation in which one of us (A. W. G.) was studying the effect of various levels of supply of nitrogen and of phosphorus, and the comparative effects of nitrate and of ammonia on the nitrogen and phosphorus nutrition of perennial rye-grass growing in pots, his attention was drawn unexpectedly one morning, March 19th, 1933, to a very striking phenomenon which had occurred overnight in the case of certain of the grasses undergoing treatment. The ends of the leaf blades of these particular grasses looked as if a whitewash brush had been drawn over them, and closer examination revealed the presence of a white substance adhering firmly to the ends.

The more detailed circumstances of the exudation were as follows. The grass had been established in the pots during the previous summer, in an artificial soil consisting of a mixture of sand with calcium bentonite and calcium carbonate, by feeding with suitable nutrient solutions coupled with very lenient cutting [Greenhill and Page, 1933]. The pots had been left untouched (except for watering when necessary) over the winter, with two to three inches of growth on them, and had been cut back to within one inch of soil level during the period March 13–16, before beginning the investigation proper. The grass at this time was making a noticeable amount of early spring growth. On March 17–18 the pots were given their respective phosphorus and nitrogen experimental treatments, which comprised three levels of phosphorus in all combinations with three levels of nitrogen, the latter being given in one series throughout as ammonium sulphate and in a second series as calcium nitrate. A uniform application of potassium sulphate was also given, while in the case of certain of the nitrogen and phosphorus treatments a second (lower) level of potash was included as well. All treatments were triplicated. The nutrients were all applied in solution by surface sprinkling, the phosphate and potash on March 17 and the nitrogen on March 18, and were followed finally on March 18 by a watering with distilled water to ensure that the salts were washed into the soil.

On the following morning, March 19, the exudation already described was observed in all those pots which had received ammonium sulphate at the highest rate of application, while it was present also, though to a much less extent, in the pots which had been treated at the intermediate rate. No exudation was present in the pots which had received the ammonium sulphate at the lowest rate, or in any of the pots which had received their nitrogen as calcium nitrate whether at high, intermediate or low rates. During the period March 19–22 the exudation showed some increase in amount in the high and intermediate ammonium sulphate pots, while very slight traces appeared also in those with low ammonium sulphate and high calcium nitrate, but no trace was observable in the intermediate or low calcium nitrate pots. No further exudation occurred after March 22.

The exudation was confined to those leaves which had been recently cut, and further, to the immediate neighbourhood of the cut ends themselves; none was observed at other parts of the leaf, or at any part of the newly growing leaves which had suffered no defoliation. Also, although the grass was watered frequently and exposed to varying weather conditions, the exudation remained unchanged and firmly adherent to the leaf blades and presented a very striking appearance for nearly a fortnight, though subsequently it gradually disappeared. The extent of the exudation did not seem to be influenced by the differential phosphate or potash treatments, nor did the exudation appear to affect in any way the growth of the grass, which proceeded in every respect quite normally and satisfactorily. Fig. 1 (taken March 24) shows typical leaf blades with and without the exudation adhering.

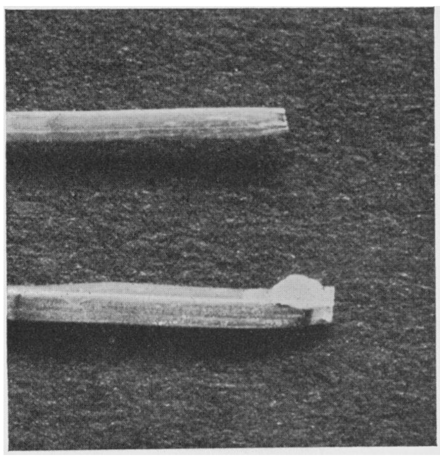


Fig. 1. J. H. 488. March 24, 1933.

Some of the exudation was removed from the leaves by means of a penknife blade on March 22 and 23 for chemical examination. The material was a white rather coarse powder which dissolved fairly readily in warm water, leaving a small residue of leaf fragments. The ash content was 10 %, and the total N by micro-Kjeldahl 14·4 %, or on an ash-free basis 16·2 %. 6·17 mg. were distilled *in vacuo* with a suspension of magnesia at 40° for 15 minutes, when ammonia equivalent to only 3·7 % of the total N passed over. The residue in the distillation flask was washed out and sufficient HCl added to bring the acid strength up to N. Boiling for 2 hours (Sachsse hydrolysis) yielded on distillation with excess of magnesia 48 % of the total N as ammonia.

The residue in the distillation flask was neutralised with glacial acetic acid and made up to standard volume. The amino-N determined by Van Slyke's micro-method was 52 % of the total N. These results showed clearly that the exudation consisted chiefly of either asparagine or glutamine. Proof that it was the latter substance was obtained by heating at p_H 8 for 3 hours at 100°, which split off 74 % of the total amide-N as ammonia [Chibnall and Westall, 1932]. Accordingly 60 mg. were dissolved in about 1 ml. of warm water, and an equal volume of warm alcohol was added. On standing typical needle-shaped crystals of glutamine separated. These were collected and recrystallised from aqueous alcohol. The material contained no water of crystallisation and gave no biuret reaction. (Found: C, 40·6; H, 6·8; N, 19·1 %. $C_5H_{10}O_3N_2$ requires C, 41·1; H, 6·9; N, 19·2 %.)

In the subsequent course of the experiment the grass in all pots was cut to within three-quarters of an inch of soil level in late April, after which the pots were again treated with nitrogen, phosphate and potash, and the plants cut back three or four weeks later. This alternating procedure of nutrient treatment and cutting after the grass had made three to four weeks' growth was repeated throughout the summer. Following each subsequent addition of the nutrient solution the exudation was again observed, but in no instances was it so striking as in the first one already described, and in no other was there sufficient material for a detailed chemical examination. The exudation on these subsequent occasions differed also from that described above in that it was not confined to the end of the cut blade, as on the first occasion, but appeared at various parts of the upper half of the blade, though always, as before, on a recently cut blade. Also, after the first two occasions, the exudation was confined almost entirely to the intermediate and low ammonia treatments.

In view of the foregoing observations in 1933, it was decided to study this spring (1934) the possible occurrence of glutamine in the leaves of perennial rye-grass actually growing in a natural pasture. Accordingly a small area of a pasture containing a high proportion of perennial rye-grass, which had been cut last

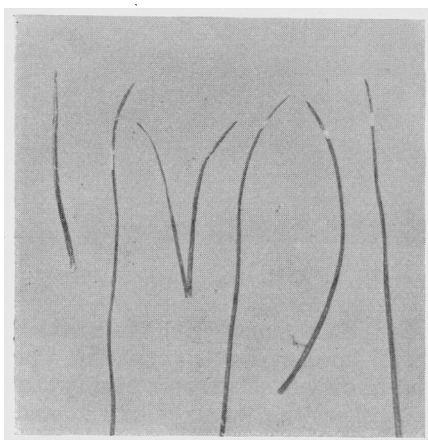


Fig. 2.

autumn but not cut or grazed since, was treated with ammonium sulphate at the rate of 2 cwt. per acre (a normal rate employed in agricultural practice) on March 23, and careful observation of the grass made daily. The perennial rye-grass at this time was making a little early spring growth. On March 26 a small amount of exudation (similar in appearance to that observed last year in the pots) was observed on a few of the leaf blades, including both those blades which had been cut last autumn and new ones which had grown since. The exudation was not confined to the end or tip of the blade, but was observed at various points over the upper half; none was observed however on the lower half of the blade. A photograph of the exudation showing its presence down the side of newly-grown blades is reproduced in Fig. 2. An examination of the exudation in the laboratory showed it to contain no ammonium salt, but it was not possible to collect sufficient material for a more detailed chemical analysis.

It should be mentioned that the phenomenon this time (in the natural pasture) would not ordinarily have been observed by the passer-by, but it was sufficiently marked to be easily noticed on looking closely into the sward. On March 27 a sample of the upper parts of the blades was cut with a pair of scissors and examined. The grass was found to contain a high concentration of glutamine, equivalent to 32 % of the total water-soluble N. No further exudation from the grass occurred, and the material exuded disappeared within a few days.

It is perhaps worthy of mention that a similar exudation was observed on some of the pasture manuring experimental plots at Jealotts Hill following an application of ammonium sulphate at the rate of 2 cwt. per acre earlier in the month, while traces were observed also on similar plots receiving only 1 cwt. per acre, but none on plots receiving no nitrogen. In other plots, however, receiving nitrogen in different forms including ammonium sulphate at the rate of 1 cwt. per acre or its nitrogen equivalent, at the end of March, no signs of any exudation were observed.

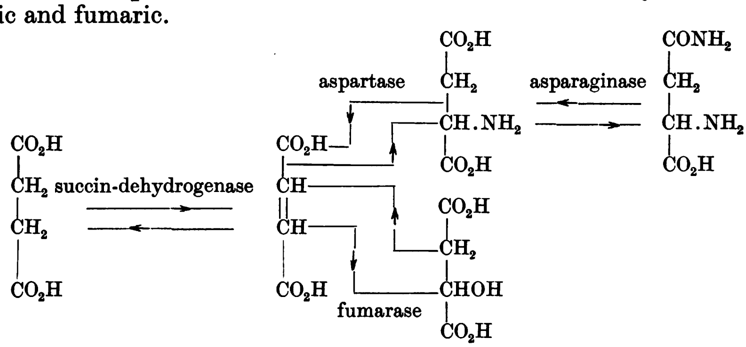
Discussion.

This rapid elaboration of glutamine from ammonium salts raises an interesting new point in connection with the nitrogenous metabolism of the plant. As is well known, chiefly from the work of Schulze at Zürich, the growth of most seedlings, especially in the dark, results in a large accumulation of asparagine (up to 27 % of the total dry weight). In certain cases, especially in the Caryophyllaceae, glutamine replaces asparagine, but it is formed in much smaller amount (up to 5 % of the total dry weight), while very occasionally both amides are formed [*cf.* Stieger, 1913].

Schulze's results were confirmed by Prianischnikoff, who showed in addition that if seedlings were grown on solutions of ammonium salts the ammonia absorbed, if in excess of immediate protein requirements, was largely stored as asparagine. Probably on account of the greater difficulty in isolating and characterising glutamine, the storage of this amide under similar conditions has not hitherto been observed, and it is therefore of particular interest to note that such storage does actually take place in the case of perennial rye-grass. Since the exudation of glutamine is most noticeable in the early spring, when the absorption of ammonium salts by the root system is usually most active, it appears that the formation of this amide is a parallel case with that of asparagine, and that it is an innocuous form of storage for ammonia present in excess of immediate protein requirements.

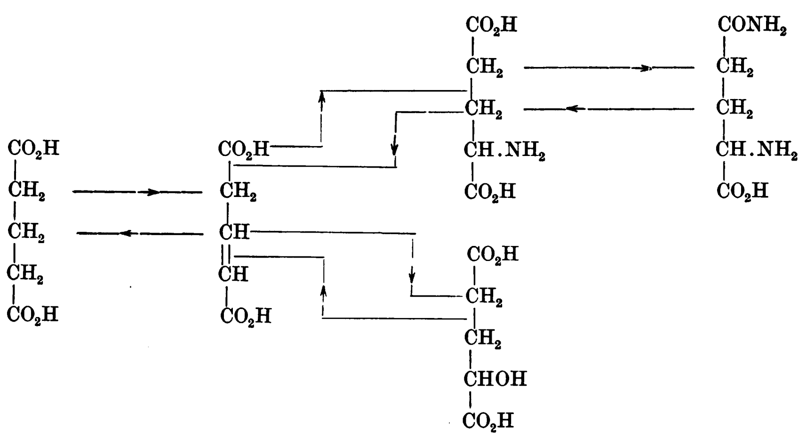
The exudation of glutamine is an unexpected phenomenon which calls for more extended study, as no similar exudation of asparagine has so far been recorded. The concentration of glutamine in the sap of the leaf cells is very much less than that of asparagine, *e.g.* in the sap of etiolated lupins, and since the solubility of glutamine in water, and therefore presumably in the cell sap, is greater than that of asparagine, the exudation cannot be ascribed to limiting solubility. It appears more probable that glutamine, even in relatively dilute solution, exercises some selective influence on the permeability of the rye-grass leaf cells, and is thereby able to diffuse outwards to the surface of the leaf, on which it is deposited in solid form by evaporation.

Now the rôle of asparagine in plant metabolism has been the subject of a large number of investigations. Schulze pointed out that the amount of aspartic acid yielded by plant proteins on acid hydrolysis was too small to permit the assumption—originally advanced by Pfeffer—that this amide was a primary product of protein metabolism. He showed that it was a secondary product of amino-acid oxidation, and it is now generally agreed that its formation, both in the case of protein breakdown and in the case of ammonia feeding through the root system, takes place from ammonia *via* the 4-carbon dicarboxylic acids, malic, succinic and fumaric.



The above scheme has, in fact, recently received striking confirmation from the experiments of Mothes [1933], who, using the vacuum infiltration feeding methods of Björkstén [1930], has been able to show that leaves of the runner bean, when fed with the ammonium salts of these three dicarboxylic acids, rapidly synthesise asparagine.

To the best of our knowledge no such searching enquiry has been made into the origin of glutamine. During the growth of etiolated seedlings or when leaves are kept in the dark, it is formed only to a limited extent, and it appears to us that there is evidence, which we shall present in greater detail in a later paper, for the assumption that it is a primary product of protein breakdown, for plant proteins yield on acid hydrolysis relatively large amounts of both glutamic acid and ammonia, while glutamine has been isolated from the enzymic digest of gliadin [Damodaran *et al.*, 1932]. Any suggestion therefore, that during etiolation the protein, fat or carbohydrate metabolism leads to the production of 5-carbon dicarboxylic acids, which, with ammonia, are the immediate precursors of



glutamine, is unnecessary. But the production of glutamine from ammonia absorbed through the root system shows that some such alternative and indirect synthesis must be possible in certain plants, and it is probable that this takes place *via* glutaconic, glutaric and α-hydroxyglutaric acids.

It is interesting to observe that both glutaric and α-hydroxyglutaric acids have been isolated from sugar beet, which also contains relatively large amounts of glutamine. Vacuum infiltration experiments to test this assumption, using blades of perennial rye-grass and the ammonium salts of these acids, are now in progress.

Summary.

Under certain conditions perennial rye-grass when treated with ammonium sulphate produces a white exudation on the upper half of the blades. The exudation consists almost entirely of glutamine.

The bearing which this observation has on the metabolism of glutamine in the plant is briefly discussed.

We should like to record our thanks to Mr R. G. Westall for analytical assistance.

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