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# Hydrocyanic Acid in Grasses.

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## I. INTRODUCTION.

The question of prussic acid in grasses is of practical as well as theoretical importance. The numerous deaths in animals caused by toxic fodder raise the subject to a vital issue for farmers. From a theoretical point of view the problem throws much light on the

physiology of the plant. Indeed, in many cases it distinctly shows the influence of environment on metabolism and also provides some hints on the possible intermediate products which could arise in the synthesis or disintegration of protoplasm. We are probably touching here on some fundamental activity of the living cell, which may enlighten us one day on the reactions leading to protein synthesis.

As in most cases of agricultural research, the theoretical aspect of the question provides the solution to the practical one. Many of the experiments mentioned in this paper have, however, been made only with the view of obtaining immediate useful conclusions for the farmer. It is perhaps this lack of academic interest in the problem which leaves us so much in the dark with respect to conditions leading to the production of prussic acid in grasses.

The publications on the subject are already abundant; a summary and a synthesis are therefore urgently called for, especially in the presence of numerous conflicting observations, which reveal once more how involved the response of the plant is towards its environment.

In our endeavour to understand some of the drastic changes observed in plants, we also far too often look for outstanding causes. Yet many of the remarkable fluctuations in prussic acid content are produced only by minute variations of climate and chemical composition of the soil. An experimental method to record such oligodynamic changes of environment is badly needed and were such a method at hand we would certainly see clearer in the problem under discussion.

In order to discuss our subject from all possible angles we shall also have to deal with a series of theories, not directly connected with grasses, the conclusion of which may however have a bearing on the plants we are dealing with. The aim of the paper is therefore not merely a compilation, but a synthesis of the existing knowledge on the question of prussic acid in grasses.

## II. A LIST OF TOXIC GRASSES.*

Prussic acid occurs in grasses either free or in the nitrile form linked to a sugar and benzaldehyde-like substances. It is doubtful whether they all contain the same glycoside; acetonecyanhydrin has however not as yet been detected in them. In most cases the plant possesses the ferment necessary to split its glycoside; sometimes the former may occur alone. No grasses are as yet known where the glycoside alone is present, yet in future such cases may still be found, if we submit the Gramineae to a severe test.

In the following list, the authors in brackets are those who have been dealing with the plant, or at least have quoted it. The first name is not necessarily that of the author who made the first investigation, because it is often difficult, if not impossible, to determine

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who started the original research. When the information is available (only from Petrie) we shall state whether the plant contains glycoside + ferment, or ferment alone.

1. *Andropogon intermedius* R. Br., native in N.S. Wales, (Petrie 1913), glycoside + ferment, strong reaction in summer.

2. *Andropogon ischaemum* Linn. Wide distribution. (Petrie 1913), glycoside + ferment; strong reaction in the summer of N.S. Wales.

3. *Andropogon micranthus* Kunth., native in N.S. Wales, (scented grass) (Petrie 1913), glycoside + ferment, but only trace, sometimes entirely free.

4. *Anisopogon avenaceus* R. Br., native in N.S. Wales. (Petrie 1913).

5. *Anthephora pubescens* Nees. South Africa. Henrici 1926. Steyn 1934); contains large amounts of prussic acid in the wilted state.

6. *Anthoxanthum odoratum* Linn. World wide. (Petrie 1913), ferment only.

7. *Aristida congesta* R. & S. South Africa. (Henrici 1926, Steyn 1934) yields prussic acid especially when the leaves are rolled.

8. *Aristida uniplumis* Licht. South Africa. (Henrici 1926, Steyn 1934), yields prussic acid when wilted.

9. *Arundo conspicua* Forsk. (*Cortaderia conspicua*) (Greshoff 1909, Pammel 1911). There is no record of this species ever having been transferred to Cortaderia under which genus it is quoted by Greshoff and Pammel.

10. *Axonopus compressus* Beauv. (*Paspalum platicaulon* Poir.) N.S. Wales. (Petrie 1913), ferment only, during part of the year.

11. *Bambusa arundinacea* Willd. Wide distribution. (Walter, Krassnosselska, Maximov and Maltschewsky 1911, Petrie 1913, Wehmer 1929).

12. *Bouteloua gracilis* (H. B. & K.) Lag. (*Bouteloua oligostachya* Torr.). Introduced from Mexico into N.S. Wales. (Petrie 1913), glycoside + ferment, maximum in midsummer in N.S. Wales.

13. *Briza minor* Linn. World wide distribution. (Couperot 1908, Greshoff 1909, Pammel 1911, Petrie 1913). Petrie's investigations in 1913 show negative results throughout the year.

14. *Catabrosa aquatica* (L.) Beauv. (Petrie gives 1908 as first date of record, but no author.) (Greshoff 1909, Pammel 1911, Petrie 1913).

15. *Chaetium bromoides* (Prest.) Benth. (Petrie 1913), ferment only.

16. *Chloris polydactyla* (L.) Sw., introduced from South America to N.S. Wales. (Petrie 1913), glycoside + ferment, throughout the year.

17. *Chloris truncata*, R.Br. (star grass), native in N.S. Wales, (Petrie 1913), glycoside + ferment, strong reaction only in January.

18. *Chloris ventricosa*, R.Br., native in N.S. Wales. (Petrie 1913), glycoside + ferment, strong reaction only in January.

19. *Cortaderia argentea* Stapf. var. *gigantea*, *rosea* and *variegata*. No record of the publication of the varieties could be found. South American Pampas grass, cultivated in N.S. Wales. (Petrie mentions 1906 as year of first record, but no author.)

    (Petrie 1913), glycoside + ferment, all varieties show strong reactions the whole year through.

20. *Cortaderia kermesiana*. (Greshoff 1909, Pammel 1911). There is no record of such a species.

21. *Cynodon bradleyi* Stent. Wide distribution. Cultivated as a lawn grass, (Steyn 1929 and 1934).

22. *Cynodon dactylon* (L.) Pers. Wide distribution. (Petrie 1913, found ferment only; Wehmer 1929, Steyn 1934).

23. *Cynodon incompletus* Nees. South Africa, N.S. Wales, (Maiden 1912, Schimmel 1913, Petrie 1913, Wehmer 1929, Steyn 1934). According to Petrie it was still doubtful in 1913 whether this grass had been introduced from South Africa, or is indigenous in Australia. According to the same author the grass contains glycoside + ferment, shows a strong reaction in winter only, loses its glycoside through desiccation while the ferment remains.

24. *Cynodon transvaalensis*, Burtt Davy. Transvaal. (Steyn 1934). Wilted specimens contain large amounts of prussic acid. (See also under climate and soil.)

25. *Danthonia semiannularis* (Labill.) R.Br., native in N.S. Wales. (Petrie 1913), glycoside + ferment, faint reaction.

26. *Digitaria eriantha* Steud. South Africa (Henrici 1926, Steyn 1934), prussic acid in wilted specimens.

27. *Dactyloctenium aegyptium* (L.) Richt. (*Eleusine aegyptiaca* Pers.). South Africa, N.S. Wales. (Petrie 1913), glycoside + ferment, reaction during part of the year only.

28. *Eleusine coracana* Gaertn. (Raybaud 1913).

29. *Eleusine indica* Gaertn. South Africa, N.S. Wales. (Petrie 1913, Raybaud 1913), glycoside + ferment, reaction during part of the year.

30. *Eragrostis pilosa* Beauv. (Petrie 1913), ferment only, during part of the year.

31. *Eragrostis pectinacea* Michx. (*Eragrostis purchii* Schrad.) (Petrie 1913), ferment only, during part of the year.

32. *Eustachys paspaloides* (Vahl) Lanza et Matti. Wide distribution. (Petrie 1913, Rosenthaler 1925, Henrici 1926, Wehmer 1929, Steyn 1934). According to Petrie, glycoside + ferment, in N.S. Wales, very strong reaction in November, faint or nil during the rest of the year.

33. *Elymus* sp. (Greshoff 1909, Pammel 1911).

34. *Echinochloa colona* Link (*Panicum colonum* L.) (Petrie 1913), ferment only, during part of the year.

35. *Festuca lachenalii* Sparm. (*Festuca poa* Kunth) (Petrie mentions first record 1908, but no author). (Greshoff 1909, Pammel 1911, Petrie 1913).

36. *Glyceria aquatica* (L.) Wahl. (*Poa aquatica* L.) (Jorisson 1884, Greshoff 1906, 1909, Pammel 1911. Petrie 1913, Guérin 1932).

37. *Glyceria canadensis* (Michx.) Trin. (*Panicularia canadensis* Michx.) Kuntze, North America (Alsberg and Black 1915).

38. *Glyceria grandis* Wats. (*Panicularia grandis* Nash), North America (Alsberg and Black 1915).

39. *Glyceria nervata* (Willd.) Trin. (*Panicularia nervata*) (Willd) Kuntze, North America (Alsberg and Black 1915.)

40. *Hemarthria compressa* R.Br. (Petrie 1913), ferment only, during part of the year.

41. *Holcus lanatus* Linn. Wide distribution (Petrie mentions date of first record 1908, but not author). (Greshoff 1909, Pammel 1911, Petrie 1913).

42. *Lagurus ovatus* Linn. South Africa, N.S. Wales. (Petrie 1913), ferment only, during part of the year.

43. *Lamarckia aurea* Moench. (Couperot 1908, Greshoff 1909, Petrie 1913). Petrie has found no trace of prussic acid in his specimens in N.S. Wales.

44. *Leptochloa decipiens* (R.Br.) Druce (Bentham refers this sp. to *L. chinensis* Nees). N.S. Wales. (Petrie 1913), glycoside + ferment, strong production of HCN throughout the year.

45. *Leptochloa dubia* (H.B. & K.) Nees (*Diplachne dubia* Scribn.) A Mexican grass introduced into N.S. Wales. (Petrie 1913), glycoside + ferment, one of the grasses that yields the highest amount of prussic acid tested by Petrie. Evolves constantly free acid throughout the year.

46. *Lolium Lamarckii* L. (Cornevin 1893. Greshoff 1909, Pammel 1911). No such species is on record.

47. *Lolium perenne* L. (Miquel 1838, Cornevin 1893, Greshoff 1909, Pammel 1911).

48. *Melica altissima* L.
49. *Melica ciliata* L.
50. *Melica nutans* L.
51. *Melica uniflora* Retz.

(Fitschy 1906, Petrie 1913, Raybaud 1913, Wehmer 1929) (Greshoff 1909 and Pammel 1911 mention genus Melica).

52. *Melica magnolii* Gren. et Godr. (Mirande 1909, Raybaud 1913).

53. *Panicum bulbosum* H. B. & K. (Petrie 1913), ferment only, during part of the year.

54. *Panicum divaricatissimum* R.Br. var. *normale* Benth. (Petrie 1913), ferment only, during part of the year.

55. *Panicum junceum* Nees. (Greshoff according to Pammel 1911).

56. *Panicum maximum* Jacq. Wide distribution. (Brünnich 1903, Greshoff 1906, Petrie 1913). Petrie has not found any prussic acid. Contains dhurrin according to Hadders and Wehmer in Klein's Handb. der Pflanzenanalyse 1932.

57. *Panicum muticum* Forsk. (Brünnich 1903, Greshoff 1906, Petrie 1913). Petrie has not found any prussic acid. Contains dhurrin according to Hadders and Wehmer in Klein's Handbuch der Pflanzenanalyse 1932.

58. *Panicum strictum* R.Br. (Bentham names this *P. marginatum* var. *strictum* Benth.) (Petrie 1913), ferment only, during part of the year.

Panicum as a genus was mentioned by Greshoff in 1909 and Pammel in 1911.

59. *Paspalum scrobiculatum* Linn. South Africa, N.S. Wales. (Petrie 1913), ferment only, during part of the year.

60. *Pennisetum latifolium* Spreng. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

61. *Phalaris coerulescens* Desf. (*Phalaris aquatica* L.) (Jorisson 1885. Jorisson and Hairs 1891, Wehmer 1929).

62. *Poa nemoralis* L. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

63. *Pogonarthria squarrosa* (Licht.) Pilger. South Africa. Henrici 1926, Steyn 1934). Wilted specimens contain prussic acid.

64. *Rhaphis gryllus* (L.) Desv. (*Andropogon gryllus* L.) Native in N.S. Wales. (Petrie 1913), glycoside + ferment, only a trace and only in winter.

65. *Rhaphis montana* (Stapf.) Phill. (*Chrysopogon serrulatus* Trin.) South Africa. (Henrici 1926).

66. *Sorghum halepense* (L.) Pers. [*Andropogon halepensis* (L.) Brot.]. Petrie indicates first record as 1903, but does not mention the author.) (Pammel 1911, Petrie 1913).

67. *Sorghum halepense* (L.) Pers. forma (*Andropogon halepensis* Sibth var. *mutica* Hack.) Native in N.S. Wales (Petrie 1913), glycoside + ferment.

68. *Sorghum verticilliflorum* Stapf. Johnson Grass. (Crawford 1906, Steyn 1934).

69. *Sorghum vulgare* Pers. [*Andropogon sorghum* (L.) Brot.] (Cornevin 1838, Abbot 1887, Palmeri 1887, Dunstan and Henry 1902, Balfour 1903, Peters, Slade and Avery 1903, Ravenna et Zamorani 1903, Ravenna et Peli 1907, Greshoff 1909, Pammel 1911, Petrie 1913, Wehmer 1929, Steyn 1934). There is a remarkable variation of the ferment. Certain leaves contain the ferment only (Petrie 1913).

70. *Sorghum vulgare* Pers. forma (*Sorghum nigrum* R. & S.) (Petrie 1913, Rosenthaler 1922, 1923, Wehmer 1929).

71. *Sorghum vulgare* Pers. forma (*Sorgrum saccharatum* Pers.) (Petrie indicates first record in 1903, but does not mention the author). [Petrie 1913, Hiltner (date?), Wehmer 1929, Steyn 1934.] Raybaud has examined 26 Sorghums, but does not mention the species.

72. *Sporobolus fimbriatus* Nees. South Africa. (Henrici 1926, Steyn 1934), prussic acid in the wilted plant.

73. *Sporobolus virginicus* Kunth. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

74. *Stipa capillata* L. (Greshoff 1909, Petrie 1913).

75. *Stipa elegantissima* Labill. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

76. *Stipa gigantea*. (Petrie 1913, mentions year of first record 1906, but no author.) There is *S. gigantea* Link and *S. gigantea* Lag. It is impossible to decide which one was meant.

77. *Stipa hystricina* Spreng. (Hébert-Hein 104, Greshoff 1906, Petrie 1913).

78. *Stipa leptostachya* Griseb. (Hébert-Hein 1904, Greshoff 1906, Petrie 1913).

79. *Stipa Lessingiana*. Trin. & Rupr. (Greshoff 1909, Petrie 1913).

80. *Stipa tenuissima* Trin. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

81. *Stipa tortilis*. Desv. (Petrie mentions the year of record 1906, but no author) (Petrie 1913).

82. *Stipa verticillata* Trin. (Bentham refers this sp. to *S. micrantha* R.Br.) N.S. Wales. (Petrie 1913), ferment only, during part of the year.

83. *Themeda triandra* Forsk. South Africa. (Henrici 1916, Steyn 1934).

84. *Trichachne insularis* Nees (*Panicum leucophaeum* H. B. & K.) (Petrie 1913), ferment only, during part of the year.

85. *Triodia flava* (L.) (Smyth [*Tridens flavus* (L.) Hitchc.] (Alsberg & Black 1915).

86. *Uniola latifolia* Mich. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

87. *Zea Mays* Linn. (Cornevin 1838, Greshoff 1909, Walsh 1909, male inflorescence; Pammel 1911, Burtt Davy 1912, Petrie 1913, Rosenthaler 1913, pistils; Wehmer 1929, Steyn 1934).

88. *Zysia pungens* Willd. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

## III. THE METHODS OF EXTRACTION.

It has been abundantly demonstrated that the prussic acid content of a grass varies considerably according to climate and season. We should therefore not be surprised to find a large discrepancy in the figures obtained by quantitative experiments. Some of these measurements are however open to criticism because they were not all carried out with a maximum care to avoid losses. Some preliminary tests should always be made to ensure that during the chosen process of extraction no acid or glycoside is eliminated.

The difficulties attached to quantitative determinations are shown by the following instance. Dowell (1919) has observed that three-quarters of the prussic acid disappears when the grass is submitted to desiccation. Petrie (1913) has discovered on *Cynodon incompletus* that while the plant is drying, the glycoside content gradually decreases while the amount of enzyme remains unchanged. The writer has however dried some grasses under controlled conditions and has found that during the process practically no acid escaped.

The above apparent contradiction may be an indication, not of elimination but of a transformation into other substances. In such a case drying would not show a discoloration of the Guignard paper, while the glycoside content may have diminished to a considerable extent. In future such possibilities will have to be borne in mind, when extractions are made.

Many authors have macerated their material in water. There is no doubt that this method involves a certain loss through two channels. The dying cells will allow a diffusion of the ferment and prussic acid is emitted as such. Moreover an aqueous solution of the acid is unstable and leads to formate of ammonia.

Bishop (1927) has submitted some of the current practices to a test and finds that distillation leads to untrustworthy results. The alcohol extraction is considered to be sound for the estimation of cyanogenetic glycosides in leaves. See also Narasimha Acharya (1933).

The following points should always be taken into account when a quantitative test is decided upon:—

1. HCN may be present in a glycosidic or non-glycosidic form.
2. During maceration the ferment may be liberating HCN.
3. The radical CN may be present in another combination from which it is not liberated by our methods of extraction.
4. Boiling the acid and its salts in water, or keeping it too long in an aqueous solution may cause loss by transformation into other substances.
5. The method may only liberate part of the prussic acid, the rest being retained by catalytic or steric hindrance.
6. The process of extraction may be creating HCN *de novo* from nitrogen compounds in the plant.

Rosenthaler (1932), in Klein's Handbuch der Pflanzenanalyse gives an extensive account of the best methods for determining prussic acid in plants and of extracting the glycoside. We need therefore not enlarge on the subject, except for the warning that each species of grass needs its own methods of extraction, which must be determined by preliminary experiments.

I wish, however, to call back to remembrance Greshoff's micro method (1889) for detecting prussic acid in plant tissues:—

"Place a freshly cut section not too thin and containing at least one layer of intact cells, in a 5 per cent. alcoholic potash solution; then transfer it after 15-90 seconds to a warm (60° C.) ferrous-ferric solution (2·5 per cent. ferrous sulphate + 1 per cent. ferric chloride) and leave it there for ten minutes and finally place it for from five to fifteen minutes in dilute hydrochloric acid (one part of conc. acid and six parts of water). A section so prepared shows minute agglomerations of Prussian blue wherever prussic acid occurred in the original section."

This method may prove useful in many instances, as a side test in doubtful cases.

## IV. THE PRUSSIC ACID CONTENT AND THE LETHAL DOSE.

There are comparatively few experiments in grasses which have been properly conducted to give a safe indication of their toxicity.

Hindmarsh (1930) has found in administering "Scheele's acid" (HCN) that the lethal dose per 1 lb. body weight of sheep and cattle is 1 mg. Avery (1903) has found that 0·4 gr. HCN *per os* will render a heifer very ill, but allow recovery. He does not state the weight of the animal. Steyn (1934) has computed from several authors (among which also Hindmarsh) that the figure for cattle is 2·2 mg. HCN per Kg body-weight intraperitoneally, and for sheep 2·2 mg *per os*.

Are we entitled, on the basis of these tests obtained by pure chemicals, to calculate the lethal dose in terms of so and so much grass, assuming we know the prussic acid content of the plant? Considering one of the experiments by Seddon and King (1930) we would feel inclined to answer in the affirmative. These authors have shown that in feeding *Acacia glaucescens* (containing sambunigrin) they confirm Hindmarsh's determination of 1 mg. per lb body-weight for sheep.

But curiously enough when feeding *pure* sambunigrin from *Acacia glaucescens*, the dose in terms of prussic acid was 2 mg. per lb. body-weight.

Petrie (1913) reports on an experiment made with *Cynodon incompletus* which was fed to sheep. The material contained 0·016 per cent. prussic acid. The lethal dose per sheep of 150 lb. was 2 lb. of grass which could liberate 0·14 grams of prussic acid. This confirms again the figure established by Hindmarsh as roughly 1 mg. per lb. body-weight.

Peters (1903) relates the case of a heifer which dropped to the ground ten minutes after having been driven into a Sorghum field. The animal was finally killed because it was obvious that it would not recover. The post-mortem showed 1½ lb. of sorghum in the paunch.

To arrive at an idea what the quantity of prussic acid involved in the last case may be, consider the maximum quantities of this substance extracted from grasses by the following authors:—

| | *Per cent.* |
|---|---|
| Dowell (1919) highest per cent. obtained on *Andropogon Sorghum* ... ... ... ... ... ... ... | 0·0514 |
| Swanson (1921) highest per cent. obtained on Sudan grass ... ... ... ... ... ... ... ... ... ... | 0·015 |
| Avery (1903) highest per cent. obtained on *Sorghum vulgare* ... ... ... ... ... ... ... ... | 0·014 |
| Pinckney (1924) highest per cent. obtained on Sorghum grown on Coloma sand with 502 lb. nitrate per acre ... ... ... ... ... ... ... ... | 0·136 |
| Willaman and West (1915) highest per cent. obtained on Sorghum (Minnesota) ... ... ... | 0·114 |

In assuming that the animal in Peters' experiment had taken Sorghum of the highest toxicity such as Pinckney had obtained, the amount of prussic acid present in the animal through the ingestion of 1½ lb. of grass would be about 0·8 gr. Assuming the animal to be about 200 lb., this would be four times the lethal dose.

But the important question here is not how much have we *introduced*, but how much can be *liberated* in such a short time. Can we assume that the cells of the grass when reaching the paunch are broken up to such an extent to liberate the lethal dose within 15 minutes? In view of the fact that the paunch is alkaline, thus not at the optimum pH for the ferment, in view of the fact also that only a small proportion of the cells are broken up within the

first hour, we may be justified in thinking that the glycoside does not liberate enough prussic acid and that therefore the latter is not the only toxic substance involved in killing the animal. The experiments both of Peters and of Petrie are open to this doubt.

The question of elimination from the animal body is all important in this connection. Prussic acid on account of its high diffusibility will readily reach the blood stream, but it will, by this same property, be eliminated very quickly. The balance between elimination and the supply from the ingested material will determine to a large degree the toxity of the dose. If the ingested material is slow to break up in the paunch, the animal will stand more than the lethal dose determined on pure chemicals.

This question that possibly another substance could be involved in these deaths should be seriously taken into account in future investigations. One way of testing the question would be to determine the prussic acid content of the material before it is fed. After the death of the animal the grass found in the paunch should be retested, to find out how much prussic acid *had actually been liberated*. The contents of the paunch would best be introduced into 95 per cent. alcohol to prevent loss during handling and transport to the laboratory. An experiment *in vitro* made with saliva and extract of the paunch would also show to what extent these juices are favourable or unfavourable to the liberation of prussic acid.

The experiment of Pease (1897) may also be mentioned in this connection. Pease claims that deaths of cattle in India from Johnson grass were really cases of nitrate poisoning. He was able to detect 20 per cent. of potassium nitrate in stems of the grass and in feeding this salt to the animals was able to reproduce some of the symptoms.

That cyanide is not toxic under all circumstances is borne out by some experiments of Loeb (1910). It is a well-known fact that the eggs of the sea urchin are killed by a pure solution of NaCl. The toxic effect of Na can be counterbalanced as Loeb has shown by sodium cyanide, a very peculiar effect, which the author tries to explain by the inhibitory action of cyanide on the oxidation.

## V. CONDITIONS IN THE ANIMAL FAVOURING OR PREVENTING TOXICITY.

In all cases of poisoning, the state of the animal previous to its eating the toxic plant must be taken into account, in cases of prussic acid poisoning more than in any others.

Swanson (1921) has shown that marked alkalinity and marked acidity have an inhibiting influence on the production of prussic acid. Thus the paunch which is alkaline and the stomach which is acid will decrease to a considerable extent the production of HCN. I have tested HCl in conjunction with pepsine and have found that the grasses crushed in a mortar emit more HCN in ordinary water than with HCl and pepsine.

The question of hunger, overstrain, bad health, thirst, drinking water before or after eating the toxic grass, should be taken into account. The effect of the toxic grass will largely depend on what

the animal had been eating before. Indeed Peters, Slade and Avery (1903) have shown that considerable doses of HCN can be given to an animal without detriment, provided it is also given an adequate amount of glucose or milk sugar. As glucose is produced by the action of ptyalin on starch, starch food may act as an antidote and so may milk. These facts may account for the many erratic results one gets in experimenting with these toxic grasses. Starch, milk and molasses should therefore be subjected to further tests for their value as antidotes or preventives.

Steyn has shown that sulphur is an excellent preventive against prussic acid poisoning (Geilsiekte). For further discussion of the question see Steyn " Toxicology of plants in South Africa " (1934).

## VI. EXTERNAL CONDITIONS LEADING TO TOXICITY OF THE PLANT.

Brünnich (1903) in quoting from a lecture by W. C. Quinell, gives the following list of " conflictng statements and theories . . . on the circumstances and conditions under which sorghum is believed to become poisonous."

1. " If sorghum is eaten in an immature condition.
2. When sorghum grows rapidly after rainfall.
3. When the plant is stunted by failure of rain or by frost.
4. When sorghum is attacked by insects during an exceptionally dry season.
5. A poisonous mould or fungus is supposed to be the medium of poison.
6. In some parts of India the plant is said to be poisonous until the rains (monsoons) are over.
7. The poisoning is attributed to the potassium nitrate which, under certain circumstances, is precipitated in the stems of the plants.
8. Physiologic changes of growth of the plant owing to climatic disturbances, such as want of rain, excess of humidity, damp cloudy weather, or prevalence of extremely variable and unnaturally high temperature."

Brünnich (1903) in his article, states that these points were submitted to an experimental examination. It would be indeed very interesting to submit them to such a test. We find, however, very little of it in Brünnich's paper.

In the above list the age of the plant and the soil conditions are not mentioned. Brünnich has dealt with them to some extent in a later part of his paper. We find further reference to these factors in the following summary by Willeman and West (1915) which states the case so clearly that I shall give it *in extenso* :—

" Maxwell states that sorghum is not fed with safety until after the seeds begin to develop: Brünnich that it should not be fed until the seeds are fully matured, Avery says that the amount of hydrocyanic acid is greater in stunted plants, while Alway and Trumbull

found that yellow stunted plants contained less of the acid than the green, vigorous plants in the same field. Maxwell believes that the amount of the glycoside is dependent on the character of the soil, soils rich in nitrogen producing plants richer in the glycoside. Brünnich, in experiments with sodium nitrate in Queensland, found that the fertilized plants contained slightly more hydrocyanic acid than those unfertilized and concluded that heavy nitrogenous soils and favourable climatic conditions increase the amount of the acid. His findings were corroborated by Alway and Trumbull. Brünnich also found that millet (*Panicum mileaceum*) behaved similarly to sorghum. Schröder and Dammann in Uruguay, report an increase in prussic acid due to the use of sodium nitrate as a fertilizer. Balfour noticed that plants infected with *Aphis sorghi* contained more hydrocyanic acid than uninfected plants."

The above remarks have been quoted with the object of showing how much still remains to be done to reach a sound view on the conditions affecting the prussic acid content of the grass.

### (*a*) Climate and Soil.

In verifying some of the above statements, Willaman and West (1915) found that nitrogen added to a poor soil may slightly increase the amount of HCN but that a fertile soil with abundant nitrogen will not show any effect on fertilization.

Pinckney (1924) " has determined HCN in sorghum plants grown in a greenhouse using three Minnesota soils low in nitrogen content and adding sodium nitrate in different amounts. The size of the plants, their colour, and prussic acid content were affected by the amount of nitrate applied ".

Although it is thus demonstrated that the soil has a marked effect on the prussic acid production, which is interesting from an academic point of view, from a practical point of view the increase is not such as to warrant any further investigations in that direction. As pointed out by Willaman and West (1915) climate is a far more important factor than soil in the production of detrimental quantities of prussic acid.

As a drastic illustration to this fact we may mention a case described by Dunstan and Henry (1902). These authors quote Bonamé who showed that the dark coloured beans of *Phaseolus lunatus* in Mauritius yield more prussic acid than the pale ones. In Burma the pale buff semi-cultivated beans contain only a trace of prussic acid. But that it is not all a question of colour and variety is borne out by Guignard's investigations, who had obtained prussic acid from white beans. He has also shown that the relation of colour to prussic acid yield in wild plants of Java is not so clearly marked as in Mauritius.

Dr. Steyn has informed me orally of a case of *Cynodon transvaalense*, where the plant, growing in moist conditions, yielded no prussic acid, whereas the plants growing on a dry ridge showed a strong reaction. A similar observation was made by Narasimha Acharya (1933) on *Sorghum vulgare*.

Climatic influence on prussic acid production is clearly demonstrated, but its effect can not as yet be foretold.

### (*b*) Diurnal and Seasonal Variations.

Diurnal variations in the production of prussic acid have frequently been recorded. Ravenna (1907) has found in sorghum an increase from morning to afternoon. Willaman and West (1916) have shown that there is a maximum at midday for the same plant. Marais and Rimington (1934) working on *Dimorphotheca cuneata* Less. found an increase in prussic acid content from early morning to noon, which they think "suggests a correlation with intense photosynthetic activity."

Narasimha Acharya (1933) working on *Sorghum vulgare* found an increase of prussic acid production from early morning to about 2 p.m., after which there is a slight decline till 6 p.m. followed by a rapid decline at night. This last author also is of the opinion that there is a correlation with photosynthesis. The writer has noticed in *Eustachys paspaloides* that there is more prussic acid in the morning than in the afternoon.

Yap (1920) has shown on sugar cane in the Phillipines that the photosynthesis of the leaves is more active in the morning than in the afternoon. They were most active from 8 to 10 a.m. and then there was a decrease from 10 a.m. to 4 p.m. This decrease after 10 a.m. does not seem to fit in with the above observations, yet one may assume that the nitrogen metabolism may lag and reach its maximum after the maximum of photosynthesis.

Much stress has been laid on the age of the plant and various workers have found marked differences in the prussic acid production as the plant grows older. The stems and leaves have been examined separately and it was generally found that the stems contain less HCN than the leaves and the leaves contain the acid in various degrees according to their situation on the stem.

Petrie (1913) in a series of grasses in New South Wales has tested the prussic acid content throughout the year and his experiments show the seasonal variations very well, and these, of course, are coupled with the age of the plant. Petrie has submitted his grasses to three tests, basing them on the assumption that the plant may contain the glycoside as well as the ferment, or the glycoside alone, or the ferment alone. The tests were carried out as follows:—

(*a*) Chloroform test (probably with Guignard paper although not stated).

(*b*) Emulsion test, in case ferment is absent and glycoside present.

(*c*) Amygdalin test, in case ferment is present and glycoside is absent.

The names are quoted *verbatim* from Petrie, " +a " denotes that case " a " gives positive results, " − " denotes negative results throughout. Here are a few cases.

| | *Jan.* | *Apr.* | *Aug.* | *Nov.* |
|---|---|---|---|---|
| *Andropogon halepensis* Sibth var. *mutica* Hack ... ... ... ... ... ... | +a | +a | +a | +a |
| *Chloris petraea* Sw. ... ... ... ... ... | +a | − | +a | +a |
| *Cynodon dactylon* Pers. ... ... ... ... | +c | +c | − | − |
| *Cynodon incompletus* Nees. ... ... ... | +a | +a | +a | +a |
| *Eleusine aegyptiaca* Pers. ... ... ... | +a | +a | − | +a |
| *Eleusine indica* Gaertn. ... ... ... | +a | +a | − | +a |
| *Lazarus ovatus* Linn. ... ... ... ... | − | +c | − | +c |
| *Paspalum scrobiculatum* Linn. ... | — | — | +c | — |
| *Penisetum longistylum* Hochst. ... | +c | − | − | − |

Investigations of the kind made by Petrie, establishing the existence of either glycoside and ferment or ferment alone should be encouraged. The grass containing the ferment only may prove dangerous when an animal has been eating another plant containing glycoside alone. A case of that nature has been described by Finnemore (1931) where *Acacia Georgina* Bailey Fv.m containing the glycosidase released prussic acid from *Eremophila maculata* containing the glycoside. We may one day come across a case where the grass supplies the ferment and another plant the glycoside. The more we know of the existence of the ferment in the plant, the better.

### (*c*) Wilting.

The health of the plant plays an important rôle in the production or disappearance of prussic acid. Wilting is a state of bad health and in this state grasses often yield relatively great quantities of prussic acid. Willaman and West (1915) have also shown that adequate water supply is usually accompanied by low, and inadequate, by high prussic acid content. The water relation of the plant, in other words, hydration and dehydration affect the amount of prussic acid produced.

Wilting is an abnormal condition of the plant accompanied by a lowered vitality. Permanent wilting is highly detrimental. The process starts by an excess of transpiration over water supply. The interstices between the cells give up their water first and the air in those interstices becomes less and less saturated. This is what Livingston and Brown call incipient drying. In heliophilous plants there may be an excess of 20-30 per cent. of water content in the cells of the leaves. In ombrophilous plants this excess is only 1-3 per cent. The releasing of the excess water produces a considerable shrinking of the cells and the leaves as a whole. The cells then lose their turgidity entirely and the shrinking protoplasm draws the cell walls inward. This drawing in of the cell walls reaches a certain limit after which the wall snaps off from the protoplasm and causes a mechanical injury to the latter.

Incipient drying is not a dangerous process and can easily be checked by introducing the plant into a moist atmosphere. Permanent wilting however cannot be immediately checked and reversed by bringing it into a moisture saturated atmosphere. The chemical changes which the dehydration has produced are too deep seated to be reversed at a short moment's notice.

The subtraction of water from the protoplasm is not a pure physical process. By the dilution and concentration of the cell contents, changes in ionisation take place which are gradually compensated by buffer action. Then certain substances may precipitate at high concentration and many a reaction will take place in the concentrated protoplasm which the less concentrated normal conditions would forbid.

Wilting is accompanied in the majority of cases by a closing of the stomata. This closing of the stomata together with the lowered vitality of the plant decreases photosynthesis to a considerable extent, there will be a lack of sugar and a lack also of oxygen, in other words, a decrease of respiratory energy. Mme. Brilliant (1924) has shown that when the water content of the leaf falls below 25 per cent. an abrupt decrease of photosynthesis is produced, lowering the process to about one-quarter of its original value.

All these facts must be borne in mind when we are trying to find a chemical relation between wilting and the enhancement of prussic acid which this state produces. We shall refer to this question again later on.

## VII. INTERNAL CONDITIONS OF THE PLANT LEADING TO TOXICITY.

The chemical and energetic processes within the cell are extremely involved and we have arrived only at a broad and summary view of the whole mechanism. Several theories have been propounded on the question of prussic acid and plant metabolism and the best we can do is to explain those theories and discuss their value in the light of most recent knowledge.

### (*a*) Theory of Goris (1921).

According to Goris the rôle of the glycoside is to protect the plant against toxic effects of certain substances like prussic acid, benzaldehyde, etc. In linking these toxic substances with a sugar the toxic effect is eliminated.

But there is an extraordinary contradiction between the formation of a glycoside for protection purposes and the subsequent releasing of the toxic substances by ferments supplied by the plant itself. The plants do not only decompose the glycoside when they are wilting, thus in an abnormal state, but the diurnal variation of the glycoside content shows quite clearly that the sugar and the aglycone are drawn into circulation again.

The idea of protection in this case is rather far fetched. The question whether a plant will make a glycoside is not so much a question of utility but a question of chemistry and catalysis. The fact that the plants containing glycosides also possess ferments to split them seems to indicate that the glycosides are storage products, whether temporary or for longer periods does not matter. Willaman and West (1915) contend that the diurnal variation shows that the glycoside in grasses is not a storage product. But the definition of a storage product is not so much based on the time for which the product is kept, but rather on the fact that an excess has been set aside for the time being. The rapid reintroduction of a storage product into circulation does not do away with the fact that it has been kept out of circulation.

Robinson (1930) has discussed this question too and shown some of its fallacies.

### (*b*) Theory of Gautier.

Gautier in 1872 contended that free nitric acid under the influence of formaldehyde produces HCN, $CO_2$ and $H_2O$. The prussic acid was then supposed to enter into long chains with formaldehyde from which Gautier derived his protein molecule.

Menaul (1920) has given this theory a test in the following way:—

"Six flasks each containing 400 c.c. of water saturated with carbon dioxide, 2 c.c. of 40 per cent. formaldehyde and 1 gr. of potassium nitrate were tested as follows:

1. Two flasks were made alkaline to phenolphthalein with sodium carbonate.
2. Two were made alkaline to methylorange but acid to phenolphthalein.
3. Two flasks were made acid to methylorange.

The flasks were stoppered and placed in sunlight for one month.

Results were as follows:—

No. 1: No HCN.

No. 2: A trace of HCN.

No. 3: 6 mg. of HCN.

"These results," says Menaul, "when considered in connection with the fact that the sap of the plant is slightly acid and that the nitrate and formaldehyde are present indicate that prussic acid may be formed in plants by the action of formaldehyde on nitrates".

It is a pity that Menaul has not made a control in complete darkness and that he has not extended the experiment to other organic acids and other aldehydes. Latham in 1886 also attached a great importance to cyanogenetic radicals in the synthesis of animal proteins.

(*c*) Theory of Meyer and Schulze, 1884.

Meyer and Schulze supposed that nitric acid through reduction, and that ammonia by oxidation may lead to hydroxylamine.

$$NO_3H \longrightarrow NH_2OH.$$
$$NH_4OH \longrightarrow NH_2OH.$$

Hydroxylamine in combining with aldehydes and ketones would form aldoximes.

$$NH_2OH + HCOH \longrightarrow H_2C : NOH.$$

These aldoximes and ketoximes were finally supposed to lead to an amino-group.

The theory does not make any statement about prussic acid. We are referring to it here because Bach based his theory on these considerations.

(*d*) Theory of Bach (1897).

Bach has carried the idea of Meyer and Schulze a step further. The nitrates are supposed to produce a certain amount of free nitric acid under the influence of organic acids. Nitric acid in the presence of formaldehyde would produce hydroxylamine. This, in agreement with Meyer and Schulze, would lead to formaldoxime.

The latter may then undergo transformation into the isomeric formamide. Formamide finally may undergo dehydration and yield prussic acid and water

$$HOCNH_2 \longrightarrow HCN + H_2O.$$

This represents the dehydration theory of Bach. That this reaction can take place had already been shown by Scholl in 1891.

Thus supposing formamide is formed in the plant, dehydration by wilting would lead to the formation of prussic acid. The theories of Gautier, Meyer and Schulze, and Bach would also account for the increase of prussic acid through an excess of nitrates in the soil and in the plant.

(*e*) Theory of Treub (1907).

The theory of Treub is much more likely to be of some value because it is held in general terms and does not attempt to describe the details of the process. Gautier has already expressed the idea that prussic acid is an intermediate step to the proteins. He has spoilt his claim to priority in a way, by putting forward too precise an idea of how he thought this process could be brought about. As these supposed reactions were purely inventions based on scanty facts the otherwise excellent idea of HCN being a step towards the proteins, was spoilt.

Treub resting within the general idea of Gautier tried to show by experiments that there is much to be said in favour of it. His principal arguments, based on observations made on *Pangium edule*, *Phaseolus lunatus*, *Indigofera*, *Alocasia* are the following:—

1. The presence of free and bound prussic acid tends to show that it is involved in metabolism.

2. The amount of HCN increases with the activities of the leaf.
3. In *Alocasia macrorrhiza* the production of HCN is limited to the green parts, which means that in those parts the nitrogen metabolism is highest.
4. In old leaves the HCN production is reduced as the metabolism is reduced.

   Before the leaves are shed they are usually free of HCN.

Treub has also suggested a modification of Gautier's theory. According to Treub the production of prussic acid is not directly dependent on energy derived from light, but is influenced by the quantity of sugar present. The reduction of nitric acid would be brought about by the sugar.

### (*f*) Objections to the Theory of Treub.

Rosenthaler (1922) has tested the theory of Treub by some experiments which were guided by the idea that if HCN is an intermediary product in plant metabolism it should be present in all plants. To prevent any source of error Rosenthaler has not used maceration for this experiment. He expelled prussic acid by a current of air after mincing the plant material. (The mincing may be a source of error.)

Out of 80 plants tested in such a way, 56 positively showed prussic acid. Rosenthaler rightly remarks that this fact in itself although favourable to the hypothesis of Treub, may not be considered as a definite proof because it does not show how prussic acid is produced and whether it is a product of synthesis or decomposition.

In order to obtain some more information on this point Rosenthaler injected an amino acid into sorghum. There is a definite stereochemical resemblance between phenylalanin and benzaldehyde-cyanhydrin, tyrosin and p-oxybenzaldehyde-cyanhydrin, valin and acetonecyanhydrin. It is also known that HCN can be obtained by the oxidation of amino acids.

Rosenthaler used tyrosin for his injection. If the idea of Treub is correct, he says, then the injection of tyrosin should induce a decrease in prussic acid. *Sorghum nigrum* was injected and showed a definite increase in HCN.

It may be recalled here that Ravenna and Zamorani (1910) have tested an injection of asparagin into Sorghum and found a decrease of HCN.

Rosenthaler's postulate that tyrosin should decrease the prussic acid content, because this amino acid resembles p-oxybenzaldehyde-cyanhydrin, rests on a very slender basis.

As a matter of fact we may *a priori* even expect the reverse, viz. than an excess of tyrosin will be transformed into p-oxybenzaldehyde-cyanhydrin and thereby increase the HCN content. But the processes involved when making a violent interference such as an injection, are so intricate that our conclusions are but wild guesses.

Rosenthaler's experiments are neither for or against Treub's hypothesis and admit of hardly any conclusion.

Oppenheimer (1925) and Stekelenburg (1931) are against Rosenthaler's conclusions.

Stekelenburg (1931) has made a series of experiments with a view to verifying the hypothesis of Treub. He has examined *Pangium edule*, *Phaseolus lunatus*, *Prunus padus* and *Prunus laurocerasus*. Stems, leaves, seeds and seedlings were submitted to a test. The method used for determination of HCN was that of Verschaffelt, with a temperature of 60° C. and maceration during 20-22 hours. The method seems open to criticism.

It would seem that Stekelenburg has drawn a series of rather sweeping conclusions from his experiments. We shall discuss their value partly here and partly under the heading of facts in favour of the theory of Treub.

Germination of *Phaseolus lunatus*: during germination the amount of HCN increases in the plant and then, as the cotyledons shrink, it decreases.

Stekelenburg concludes from this experiment that the cyanogenetic glycosides function as carbohydrate reserves. The releasing of HCN is not necessary because in his opinion the plant is drawing enough nitrogen from the soil. He points to the fact that Ravenna has found the same phenomena of increase followed by a decrease on a soil devoid of nitrogen. According to Stekelenburg the experiment would prove that HCN is derived from some organic compound.

But the experiment does not warrant any such far-reaching conclusions and can be well interpreted in favour of Treub's hypothesis.

Buds and stems of *Prunus padus* and *Prunus laurocerasus*. The prussic acid content was measured before budding and then after, on cut twigs kept in the dark, cut twigs kept in light and on twigs still attached to the plant in light.

In *Prunus padus* the HCN of the stems remains practically constant. There is an increase of HCN in the buds under most of the above-mentioned conditions. In *Prunus laurocerasus* the etiolated buds (cut twig in the dark) showed a decrease, while the others manifested an increase.

The constancy of the HCN in the twigs and the increase in the buds tend to show that there is no migration of the acid. The facts do not in themselves support the idea which Stekelenburg here again emphasizes that cyanogenetic glycosids are storage products. In one case darkening had no effect and HCN increased, in the other it had an effect and decreased the prussic acid content.

*Leaves.*

During the day *Prunus laurocerasus* increases its HCN and maintains it constant during the night. There is no migration. The decrease starts in the dark, after the starch has disappeared. Leaves floating in 1 per cent. glucose sol. increase their HCN, whereas in pure water there is a decrease.

These results again are not in themselves of any support to the theory of reserves.

The more important fact which arises out of these experiments is that HCN increases when no nitrogen is offered to the plants. Yet the experiments are not of a conclusive character because the author has overlooked the possibility that the leaves may contain a considerable reserve of nitrates and they need very little to keep alive.

The influence of nitrates was tested in the following way. Leaves were floated on 0·1 per cent. nitrate solution and showed a decrease of HCN. When glucose was added an increase was noted equal to the increase above when the sugar was given alone. A 1 per cent. asparagin solution showed a decrease but in conjunction with sugar showed a marked increase. The author concludes that nitrates are not necessary for an increased HCN production. This conclusion meets with the same objection as above. If the plant contains enough nitrates in reserve, an excess will only be detrimental. The administering of sugar may have another effect. The effect of asparagine is still mysterious and no conclusion can be drawn from it.

Stekelenburg concludes from his experiment that HCN is not the first visible assimilation product of nitrogen, that therefore the hypothesis of Treub is erroneous. He further contends that HCN is a by-product derived from higher nitrogen compounds and has no importance in the N-metabolism. Transport of HCN does not take place. HCN may have a certain value as nitrogen reserve.

While we agree that the cyanogenetic glycosides are temporary storage products, we cannot subscribe to the author's conclusions with respect to the theory of Treub. The experiments in themselves, though a valuable contribution, do not carry that element of conviction, nor are they to the point. They simply do not permit of any definite conclusion with respect to the hypothesis of Treub and may, as we will show, just as well be used to confirm it.

### (*g*) Facts in Favour of the Theory of Treub.

Greshoff, Ravenna, Dunstan and Henry, Oppenheimer and many others are in favour of the Theory of Treub.

To Greshoff's mind (1906) the wide distribution of prussic acid throughout the plant world, in a large number of families, is an indication of the importance of the acid in those cases where it is linked with acetone (acetone-cyanhydrin); it may possibly be an intermediate product in protein synthesis, in such plants as *Panguim edule*, *Linum usitatissimum*, and *Phaseolus lunatus*. He thinks, however, that in such cases where the acid is linked with benzaldehyde such importance may possibly not be attached to it. But it is difficult to see why in the one case prussic acid should be part of metabolism and why in the other it should not. The combination which the excess prussic acid will undergo, depends on whether benzaldehyde or acetone is present and these products are in themselves no indications of the purpose for which the acid is produced.

Ravenna (1912) also accepts the theory of Treub and thinks that HCN is a stage from nitrates over the amines to proteins. He thought that this idea was strengthened by the fact that asparagin injected into the plant decreases the amount of HCN. But Czapek points out that aromatic substances produce the same decrease so that this argument is of as little value as the one forwarded by Rosenthaler for the opposite effects.

Ravenna (1912) also pointed out the fact of diurnal variation and thinks that prussic acid is produced by nitrates and carbohydrates in the presence of light. He also showed that the maximum prussic acid is produced in the leaves.

The diurnal variations in the prussic acid content of grasses is a striking fact which speaks in favour of Treub's theory. These rapid variations show that the excess acid is temporarily stored away and very rapidly also brought back into circulation. The theory is also supported by observations made in the shade and in the sun. Dr. D. G. Steyn has informed me orally that at 8 a.m. *Cynodon transvaalense* showed a high prussic acid content in the sun and a few yards away, in the shade showed none. Here evidently the higher activity in the sun will naturally produce an excess.

The increase of HCN by an abundant supply of nitrogen fertilizers also is in favour of Treub's theory.

We may also derive some arguments in favour of the theory from our consideration on wilting. By the lowered photosynthesis and the lowered supply of energy for the endothermic processes, the creation of new proteins will be very slow. Supposing this lowered energy supply does not affect the intermediate products as much as it does the end products, wilting would inevitably produce an excess of prussic acid if this substance is involved in the metabolic process., Henrici (1926) has considered this as a possible explanation too.

On the other hand we may, under normal circumstances, increase prussic acid if by an excess supply of nitrates we increase the rate of production of the intermediate products.

The process could be summarised as follows on the basis of Treub's idea.

1. *Normal Process.*

| | | | | |
|---|---|---|---|---|
| Nitrates | → | HCN. | → | proteins |
| Photosynthesis | → | Sugar | → | Respiratory |
| Respiration | → | Oxygen. | | Energy |

2. *Wilting.*

Decrease of respiratory energy; decrease of protein synthesis therefore excess prussic acid through accumulation of intermediary products.

3. *Normal energy supply but excessive supply of nitrates.*
Result excess HCN.

4. *Excessive activity in the sun.*

General increase of activity, increased transpiration, increased circulation. If the increase in circulation is higher than the increased protein synthesis, the case would correspond again to No. 2 or No. 3, accumulation of intermediary products.

Apart from the facts that Treub himself has pointed out, we may draw some arguments in his favour from the experiments of Stekelenburg (1931).

Seedlings and buds are known for their very high activity; thus naturally one would expect a high prussic acid content. It would be rather strange if those two would produce HCN and sugar for storage products. The facts seem to point rather in the other direction that an excess of sugar and HCN produced during high activity, meet and forcibly have to combine to form the temporary glycoside.

That darkening of the buds has no effect can be expected because these organs generally carry an excess of sugars and other nutrient substances, so that the lessening of photosynthesis will not affect them so much. In *Prunus laurocerasus* the darkening of the buds had a decreasing effect in Stekelenburg's experiments, so that all depends on the amount of reserves they contain.

The darkening effect on adult leaves is very marked, as can be expected, because they have very little reserves and are dependent on direct photosynthesis. As Stekelenburg points out the decrease in the dark starts when all the starch is used up, that means when the respiratory energy goes down. This is borne out by the experiment where leaves are floating on 1 per cent. glucose solution. The sugar here is taken in, not for the purpose of storing it, but for respiration and synthesis. An excess may yet be stored. But the abundance of sugar enhances respiration and thus the general activity of the plant.

That the nitrates do not produce an increase of HCN when offered to the leaves proves nothing. The leaves may contain an excess of nitrates already and offering them more will not help.

All experiments of Stekelenburg tend to show that active photosynthesis is coupled with high prussic acid content, confirming thus other experiments on the same factors.

Taking into account all facts, even those of Rosenthaler showing how widespread HCN production is in plants, we would feel inclined to grant the hypothesis of Treub, the title of a good working hypothesis.

This does not wholly do away with the possibility of creating prussic acid by other means, such as those described by the theory of Bach or shown by the experiments of Plummer (1904) who obtained prussic acid by oxidation of albumins. Aslander (1928) has shown that cyanides decompose rapidly in the soil and he ascribed the action to micro-organisms. Emerson [quoted from Czapek (1922)] has discovered that *Bac. pyocyaneus* digests proteins in an acid medium with the production of HCN.

## VIII. EFFECT OF FREE PRUSSIC ACID ON THE PLANT.

The detrimental effect of prussic acid on the respiration of an animal is well known. It is ever so much more amazing that the abundance of that acid in plants does not seem to be injurious to plant cells.

In conjunction with the question of respiration and the effect of HCN, we should briefly recall the two theories which have a bearing on the question.

The theory of Warburg centres around the activation of oxygen which to his mind is done with the help of iron. The iron, according to this theory, in passing from a lower to a higher valency would be capable of producing peroxides of ever higher oxidising power. In this theory the inactivation of respiration by HCN would be explained in assuming an inactivation of the iron (ferri-form) by the prussic acid. If it were so, the plant does not seem to suffer much from such inactivation, whereas the animal is killed very rapidly.

According to the idea of Wieland, oxygen does not need to be activated. His theory centres around the activation of hydrogen brought about by dehydrogenation. Although for the chemist and with respect to the end products, direct oxidation is equivalent to dehydrogenation, yet for the organism they are not the same because different means are needed to bring them about. However excellent the idea of dehydrogenation may be, Wieland is at a loss to explain the inactivation of respiration by HCN. He tried to escape the difficulty by saying that prussic acid attacks the catalase and that the organism thus suffers from an excess of peroxides. The argument, however, is very weak.

In considering the considerable quantities of prussic acid produced, one wonders why the respiration of the plant is not impaired. The sorghums never seem to be free of the acid; if it is a part of the ordinary metabolism the plant organism can never be devoid of it. Some plants emit the acid freely, they live in an atmosphere constantly containing prussic acid, like *Nerium oleander*, and yet do not seem to suffer.

Yet a certain excess may still be harmful. Brinley (1927) has tested the effect of HCN on living cells. The acid seems to enter the cell as a molecule and not as an ion, although in water it dissociates to a slight degree. The rate of recovery of *Elodea* cells after having been placed in a dilute solution of HCN is a linear relation. The toxicity of HCN to the root hairs of *Limnobium* results in a uniform curve, suggesting a unimolecular reaction. HCN seems to increase the permeability of the cell membrane. (Quoted from Biol. Abstracts 1930, No. 7279.)

Hassebrauk (1928) has tested the effect of HCN on the maturity of seeds, among other plants also *Dactylis glomerata* and *Anthoxanthum odoratum*. The seeds were gassed with HCN and the effect proved favourable to after ripening and germination (quoted from Biol. Abstracts 1929, No. 17940)

Boresch (1929) undertook experiments to test whether the presence of HCN was related to the dormancy of the buds and to the breaking of their rest periods. No broad relationship was found, but " yes " would answer the question better than " no ". (Quoted from Biol, Abstracts 1932, No. 6590.)

Cotte (1914) has shown how different plants vary in their sensitivity to HCN. This author tested *Triticum*, *Tropaeolum minor* and *Ricinus communis*. For the experiment the plants were kept in an airtight compartment of 0·64 cubic meters. These are his results:—

| | |
|---|---|
| 8 gr. HCN acting during 1 hour: | Triticum not affected.<br>Ricinus not affected.<br>Tropaeolum not affected. |
| 10 gr. HCN acting during 1 hour: | Triticum slightly affected but surviving.<br>Ricinus slightly affected but surviving.<br>Trapaeolum no effect. |
| 15 gr. HCN acting during 1 hour: | Triticum strongly affected, some dead after 27 days.<br>Ricinus strongly affected, some plants killed.<br>Tropaeolum slightly affected. |
| 25 gr. HCN acting during 1 hour: | Triticum completely destroyed.<br>Ricinus completely destroyed.<br>Tropaeolum slightly affected but recovered. |
| 25 gr. HCN acting during 2 hours: | Triticum completely destroyed.<br>Ricinus completely destroyed.<br>Tropaeolum injured but survives and flowers. |

Tropaeolum shows thus a very high resistance towards the effect of prussic acid. Triticum and Ricinus are less resistant but the doses they can stand are still amazing.

No correlation could be established between anthocyan and prussic acid. It seems, however, according to my own experiments, that those parts of the leaves containing anthocyan produce more prussic acid than the green parts of the same leaf. The remarks of Henrici on this point are not quite clear.

## IX. PRUSSIC ACID IN GLYCOSIDIC OR NON GLYCOSIDIC FORM.

Willaman (1917) thinks that prussic acid exists in a glycosidic and a non-glycosidic form. Dowell (1919) contends that his experiments do not show the presence of the non-glycosidic HCN. But Willaman may be right with the restriction that the non-glycosidic form cannot last very long and if there is enough sugar present the glycoside will be immediately created.

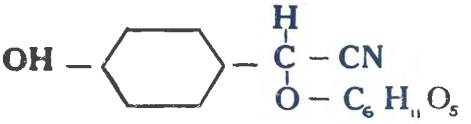
Narasimha Acharya (1933) thinks that there are at least three forms in which prussic acid is present:—

1. "Free prussic acid" formed by enzymic hydrolysis of "labile" prussic acid, destroyed by 10 per cent. sulphuric acid and steaming.
2. "Labile prussic acid" liberated by simple steaming. Destroyed by 10 per cent. sulphuric acid.
3. "Bound prussic acid", liberated by enzymic action, destroyed by heating and 10 per cent. sulphuric acid.

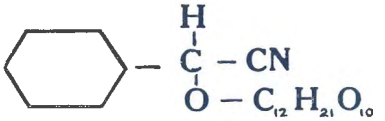
This is an interesting point which would deserve further investigation especially in view of throwing some light on the theories of prussic acid production as an intermediary stage of plant metabolism.

The glycosides so far isolated from grasses are amygdalin and dhurrin. (Dunstan and Henry 1902.)

The chemical composition of dhurrin is as follows:—



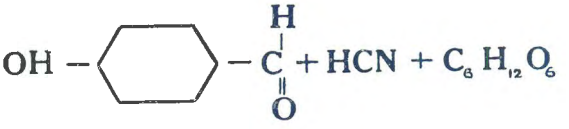
It will be useful to compare it with the well known amygdalin.



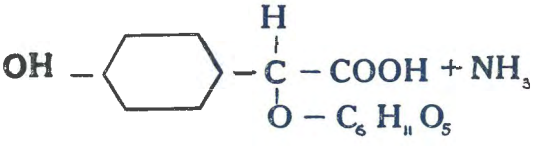
There is an extremely close resemblance between the two substances. The main difference lies in the sugars, dhurrin being coupled with a monosaccharid while amygdalin is linked up with a disaccharid. Moreover, dhurrin possesses a hydroxyl in para-position.

The effects of acids, emulsin and alkalis on dhurrin are the following:—

1. Hydrolysis by acids and emulsin:



2. Hydrolysis by alkalis:



producing dhurrinic acid and ammonia.

Considering the first fact, one would assume that HCl must forcibly increase the liberation of prussic acid. But as Swanson (1921) and the writer have found, the reverse is the case, HCl has an inhibiting influence when acting on the plant. This may be due to an effect on the ferment; the case needs some closer investigation. The influence of alkalis on the plant tends also to diminish the HCN. This may also be due to a direct effect on the ferment which works at pH 4·6, or to the above hydrolysis by alkalis.

Thus these two reactions need some more careful investigations, when considering the effect of the alkalinity in the paunch and the acidity in the stomach.

Judging from the link between the nitrile -CN and the rest of the molecule, one would *a priori* admit that the ferment which is capable of splitting off HCN from amygdalin will be capable of doing so also from dhurrin. This has been amply verified. Yet it should not be overlooked that the presence of the hydroxyl in dhurrin may, under certain circumstances, render the action of emulsin difficult, if not impossible.

The ferment that is capable of splitting amygdalin into its components is the well-known emulsin. It should be recalled at this juncture that emulsin is by no means a pure ferment and is composed of a series of components which are difficult to isolate. Its first component is an amygdalase which splits the disaccharide (called amygdalose) into glucose and d-benzaldehyde-cyanhydrin-β-glucoside; the latter substance is prunasin. The first component of emulsin will not come into action for dhurrin, because as stated it only possesses a monosaccharid and could be called an oxy-prunasin.

The second component of emulsin, a prunase, splits off glucose from prunasin and will probably do the same for oxy-prunasin with the restriction mentioned above.

The third phase of fermentation is supposed to be performed by an oxynitrilase which would split off HCN from the cyanhydrin. The question how this oxynitrilase acts and whether it is a real ferment or not is not as yet settled, the reader will find a detailed discussion of the question in Oppenheimer: "Die Fermente."

The nature of the ferment present in the grasses which is capable of splitting dhurrin, has not received enough attention. Is this dhurrase in any way similar to emulsin in that it is a mixture of prunase, oxynitrilase and other ferments? If it were only an oxynitrilase its ferment nature may be doubted on the same grounds as that of the same component of emulsin. Attempts should be made to isolate this ferment and investigate it in all fermentative activities. There is no doubt of the existence of this ferment. In most cases, to obtain HCN, the leaves need just to be crushed or treated with chloroform vapour, so that ferment and glycoside may diffuse and react. Dunstan and Henry (1902) say that provisionally the ferment of *Sorghum vulgare* can be considered identical with emulsin.

## X. PRUSSIC ACID AS AN ORGANIC COMPOUND.

Prussic acid is a tautomeric substance which occurs in two forms.

$$H-C\equiv N \text{ and } H-N\equiv C$$

When replacing the hydrogen in these two isomers by organic radicals we obtain from the first, the nitriles, and from the second the isonitriles. The latter seem to be much more toxic than the former.

The salts of the acid are generally a mixture of both isomers and are difficult to separate.

By hydrolysis the nitriles lead to an organic acid, $R-COOH$ and the isonitriles to an amine $R-NH_2$. A mixture of these two derivatives of prussic acid has thus already an amphoteric character.

We may possibly have a clue here to the second toxic substance which accompanies prussic acid, to which we have alluded, in the beginning. If prussic acid and its derivatives are in some way linked up with the protein metabolism it is very likely that both nitriles and isonitriles will be produced. They have both been found in plants. One of them only seems to form the glycoside, viz. the nitrile. The isonitriles may thus form a series of toxic substances which are not all detected by our prussic acid tests. This point, therefore, deserves serious investigation.

Some of the properties of prussic acid may interest us here with respect to precautions to be taken during extraction.

An aqueous solution of HCN is unstable and leads to ammonium formate. The pure acid is rapidly decomposed by concentrated H Cl, with production of formic acid and ammonium chloride.

The first fact should be borne in mind when keeping a solution of HCN after extraction. The second is important with respect to the influence of HCl on the production of prussic acid by the plant. Although the concentration of HCl used in our investigations is very low, yet the decomposition of HCN may not be negligible. The influence of hydrochloric acid on the prussic acid production seems to be very complex. Although it is capable of hydrolysing dhurrin, its probable influence on the ferment and its direct effects on prussic acid itself, decrease the production of HCN to a very large extent.

The salts of prussic acid undergo decomposition when boiled in an aqueous solution. They produce a formate and ammonia. This fact should also be taken into consideration in all quantitative extractions.

## XI. EXPERIMENTS.

All investigations described below have been made with the help of Guignard paper. This paper is prepared as follows:—

> 5 grams of sodium carbonate and 0·5 gr. of picric acid are dissolved in 100 c.c. of water. Strips of filter paper are dipped in this solution and then air dried. When the strips are still damp they are introduced into a well stoppered test tube. The test should always be made with a slightly damp paper.

The above test is a qualitative reaction and gives no information as to the origin and actual quantity of the HCN liberated. It may, however, be considered, to a certain extent, as a quantitative test when we observe the degree of darkening of the paper and the time it takes to reach a certain shade.

(*a*) EXPERIMENTS ON *Sorghum verticilliflorum*.

1. Leaves not crushed + chloroform—very rapid reaction, the Guignard paper turns violet within 7 minutes.

2. Leaves not crushed without chloroform—no sign of prussic acid even after 24 hours.

   These two experiments distinctly point to a fermentative process and show that in this case, at that particular instant, all prussic acid was glycosidic.

3. Crushed leaves, without chloroform—paper turned more brown than violet, but darkens just as much as No. 1, though slower.

   The crushing brings the glycoside and the ferment together.

4. Crushed leaves + HCl (0·1N HCl added just to cover the crushed leaves)—positive reaction but weaker than No. 3.

   At 39° C there is an increase in the production of HCN. Probably this temperature drives out a certain amount which was dissolved.

   This confirms the findings of other authors on the inhibitory effect of HCl on the prussic acid production.

5. Uncrushed leaves + HCl—(0·1N HCl added just to cover the leaves)—no sign of prussic acid after 24 hours.

   Heating to 39° does not help.

   This is rather strange in view of the fact that dhurrin can be hydrolysed by HCl.

6. Base of stem (containing much anthocyan) + chloroform—strong positive reaction within 7 minutes.

7. Base of stem crushed, without chloroform—slight sign of prussic acid after 4 hours; stronger after 24 hours, but not as strong as No. 6.

   The crushing in this case does not seem to bring the glycoside and the ferment so intimately into contact as chloroform does by affecting the permeability. The crushing may bring about a series of reactions, which hamper the fermentation of the glycoside.

8. Old stems + chloroform—positive but very weak even after 24 hours.

9. Runners + chloroform—positive but very weak even after 24 hours.

10. Runners crushed, without chloroform—positive but very weak even after 24 hours.

11. Leaves that have been lying on the table for 1½ hours tested again with chloroform—very strong reaction.

12. *Hay.*—Six open tubes containing fresh grass with Guignard paper (no chloroform) were introduced into a desiccator containing $H_2SO_4$ conc. This is a rapid hay production. The grass was tested after 2 days. The Guignard paper was only slightly tinted by escaping prussic acid. This shows that the process of drying does not in all cases result in an emission of prussic acid, although by transformation of HCN into other substances it may result in a loss.

    The tubes were tested by heating to 52° C with the following liquids (leaves partly immersed):—

    (*a*) 1 tube dry grass + dilute HCl—strong positive.

    (*b*) 1 tube dry grass + dilute ammonia—strong positive.

    (*c*) 1 tube dry grass + distilled water—strong positive.

    The hay thus contains a considerable amount of prussic acid, the liberation of which under these particular conditions does not seem to be hampered by acidity or alkalinity.

    Hay from the same plant was retested 5 months later, but no longer showed signs of prussic acid.

### (*b*) Experiments on *Eustachys paspaloides.*

Four tins each containing a plant of *Eustachys paspaloides* were brought to my laboratory. The leaves were immediately tested and showed strong production of prussic acid. Four days later there was no longer any sign of prussic acid. As the plants had been transplanted they were very weak and probably used up their prussic acid in urgent metabolism.

The following tests were made on the grass:—

1. Spikelet.
    (*a*) With chloroform—no sign of prussic acid.
    (*b*) Without chloroform—no sign of prussic acid.

2. Leaves.
    (*a*) With chloroform—strong production of prussic acid.
    (*b*) Without chloroform (21° C)—no sign of prussic acid.

        Here again there seems to be no non-glycosidic prussic acid.

        The experiments with higher temperatures were made in the following way: The grass blades were cut to the length of 6 cm. and introduced into an 8 cm. test tube. At the bottom of the tube 0·5 cm. of water were placed and a Guignard paper was suspended inside by the help of the stopper. This test tube was then immersed in a water bath of the required temperature.

    (*c*) Without chloroform 45° C.—no sign of prussic acid after 2 hours. The tubes were left overnight at ordinary temperature. In the morning after 18 hours the paper was dark brown from the liberation of prussic acid.

(*d*) Without chloroform at 70° C., Guignard paper turns brown after 5 minutes. This high temperature seems to have much the same effect as chloroform on permeability. If the plants, having remained at 70° C. for some minutes, are tested with chloroform at ordinary temperature, there is no production of prussic acid. Most probably the ferment has been killed at 70° C.

(*e*) Without chloroform 59° C. This is probably the temperature of wilting grass on a hot day. The grass kept at 59° C. for 10 minutes shows a strong production of prussic acid. If the same grass is again tested at ordinary temperature with chloroform, there is no sign of prussic acid. But after 16 hours there is a slight recovery. The ferment still seems to be injured but not to the same extent as at 70° C.

*Stems.*

Tested with chloroform—no sign of prussic acid even after 48 hours.

A normal solution of HCl does not produce any prussic acid on uncrushed leaves after 2 hours at ordinary temperature.

If old plants devoid of HCN are cut down and left to grow, the young leaves show HCN again.

The parts of the leaves containing anthocyan show a stronger production of prussic acid than the purely green parts of the same leaves.

Leaves collected in the morning produce more HCN than those collected in the afternoon. This confirms the observations of many other investigators.

*Hay Production.*—Four tubes with fresh leaves were kept in a desiccator over sulphuric acid. A strip of Guignard paper was introduced into each tube. There was no sign of prussic acid during drying. The tubes were kept 4 days under these conditions. After that the following tests were made:—

1. The hay was moistened and heated to 70° C. in a water bath. Strong reaction of prussic acid.

2. The hay plus 1 per cent. HCl acid covering 1 cm. of the base of the 6 cm. leaves left at ordinary temperature. A slight but very distinct amount of prussic acid is produced within 24 hours.

3. The hay plus 1 per cent. HCl heated to 70° C. produces a slight amount of prussic acid.

   Here we have the inhibiting effect of HCl again in a very marked degree.

4. Hay alone heated dry to 70° C. No sign of prussic acid. After addition of a few drops of water at that temperature the Guignard paper immediately showed the reaction.

The leaves of *Eustachys paspaloides* were squashed in a mortar and pounded with sand in order to break them up. They were then treated with standard acetate pH 4·6 which is the optimum pH for emulsin.

Emulsin was extracted from almonds.

1. The crushed leaves + standard acetate in a test tube showed no signs of prussic acid.
2. The crushed leaves + standard acetate + emulsin showed strong signs of prussic acid.

The experiment with emulsion is often deceptive because the ferment is a paste which does not allow the gas to escape so easily.

Crushed leaves (without standard acetate) were tested with HCl and pepsin. This was made to reproduce the conditions in the animal's stomach.

1. Crushed leaves without chloroform—showed signs of prussic acid after 10 minutes.
2. Crushed leaves with chloroform—strong production of prussic acid.
3. Crushed leaves + 1/1000 HCl (acid just covering the leaves)—no sign of HCN at 18° C., but distinct sign at 36° C.
4. Crushed leaves plus 1/1000 HCl plus pepsin—weak sign at 18°, distinct increase at 36° C.

As compared with the production of HCN under chloroform, the production of acid under pepsin and HCl is negligible. This is another proof of the inhibitory effect of HCl.

An illustration of the variation in the prussic acid content on two consecutive days is given by the following experiment. Five plants were examined and showed the following reactions:—

No. 1.—No sign of HCN.
No. 2.—No sign of HCN.
No. 3.—Strong production of HCN.
No. 4.—Weak production of HCN.
No. 5.—No sign of HCN.

Twenty-four hours later the situation was as follows:—

Nos. 1, 2, 3 and 4.—Weak production of prussic acid.
No. 5.—Strong production of prussic acid.

Some of my observations tend to show that a cold wind will considerably reduce the production of the acid.

### (*c*) Extractions.

The following experiments may serve as an illustration of the difficulties and pitfalls in the process of extraction. They may be useful in future investigations to avoid sources of error.

*Experiments on* Sorghum verticilliflorum.

Plants tested on their arrival with chloroform—positive, strong.

1. Alcohol extract 95 per cent. plus a small amount of calcium carbonate.

Tested the extract by suspending a Guignard paper above it in a test tube—

(*a*) cold—negative;

(*b*) warm—negative.

Thus the 95 per cent. alcohol extract, although it contains both ferment and glycoside, will not allow of any liberation of HCN.

2. Evaporated alcohol on water bath (70° C.) and residue taken up with water.

Test of the solution:—

Cold.—Negative.

Warm, 61° C.—Positive.

The method so far is safe and can be employed without fearing any loss of the acid by evaporation.

3. Alcohol extract 42·5 per cent. plus calcium carbonate.

This extract was tested:—

(*a*) During extraction—strong production of HCN.

(*b*) Cold after extraction—strong production of HCN.

(*c*) Warmed again after 12 hours, 60° C.—strong production of HCN.

Thus alcohol of a lower concentration is not at all safe for extraction purposes, because the losses during the process are far too high.

To obtain some information about the influences of metals on the production of HCN the following experiment was made:—

100 c.c. of extract No. 3 was treated with 50 c.c. 50 per cent. ammonium oxalate.

The ammonium oxalate precipitates Fe, Mg and Ca. The treated solution was filtered and the filtrate examined. At 60° C. the Guignard test proves positive.

The subsequent addition of $FeSO_4$, $MgSO_4$ and $CaCl_2$ makes no difference. $MnSO_4$ and $AlCl_3$, however, have a distinct inhibitory effect.

Purification with lead acetate can be done in two ways. It can either be added to the alcohol extract No. 1 or to the extract No. 2.

In the first case when lead acetate is added to the alcohol (95 per cent.) extract, there is a positive Guignard reaction before filtering. After filtering the filtrate shows but a weak sign of prussic acid. When filtrate and residue on the filter are brought together again, there is no reaction on Guignard paper.

It may be concluded from this experiment that lead acetate brings down the ferment, which being concentrated at the bottom of the flask reacts strongly on the glycoside for a short while. But the precipitation being accompanied by denaturation of the ferment, the fermentive action soon stops.

This seems to be borne out by the second experiment. When lead acetate is added to extract No. 2 (water) and lead is eliminated by ammonium oxalate, the following results are obtained:—

(1) At room temperature –

(*a*) filtrate- negative;

(*b*) filtrate + emulsin – positive.

(2) At 65° C.—

(*a*) filtrate—Guignard positive but weak;

(*b*) emulsin alone –negative.

(*c*) filtrate + emulsin—Guignard positive strong.

This shows quite clearly that lead acetate precipitates the ferment. If the ferment is subsequently replaced by emulsin the positive results are obtained again.

The experiments also demonstrate that both the ferment and the glycoside are soluble in alcohol, but that most probably the alcohol itself or another substance equally soluble in alcohol prevents them from reacting. This may serve as a basis for finding an antidote for poisoned animals. The substances which are dissolved in 95 per cent. alcohol are fats, essential oils, phytosterines, phosphatides, fatty acids, glycosides, resins, tannins, chlorophyll, etc. Some of these substances should be tested by veterinarians to see whether they are of any use under the conditions of the digestive system of the animal to prevent further liberation of prussic acid. In all these experiments on animals there should be constantly borne in mind the statement made at the beginning of this paper, that prussic acid may not be the only toxic principle. We should also not forget that in the paunch there is a strong bacterial action which may increase the production of HCN.

The glycoside is not very soluble in ether. This was shown by the following experiment. Liquor No. 2 was shaken out with ether and the two liquids separated. The ether was left to evaporate and the residue taken up with water. This solution treated with emulsin gave a faint reaction. The liquor which had been separated from the ether was freed from the latter and was tested with emulsin. The reaction proved very strong.

I have only tried one adsorbent in an endeavour to separate the glycoside from the ferment. Polyaluminium hydroxide was used on the alcohol extract. The solution was filtered and the filtrate evaporated and then taken up with water. It proved positive to Guignard paper showing that neither glycoside nor ferment was extracted to any noticeable extent. Moreover, the polyaluminium hydroxide when added to this last solution had an inhibiting effect.

An acid water extract was also made with tap water + HCl to make it 0·1 normal. The extract alone heated to 65° C. shows strong production of prussic acid. When this solution is treated with lead acetate it becomes strongly fluorescent. The filtrate and the precipitate both show the fluorescence.

The precipitate was separated from the filtrate by filtration and the residue on filter tested with emulsin which proved positive. The filtrate was also tested with emulsin and proved positive. This is good evidence that the glycosid partly goes down with the lead precipitate and partly remains in solution. This fact should be borne in mind when lead acetate is used to clear an extract. It shows that the method of Dunstan and Henry can not be used for reliable quantitative determinations of the glycoside.

## SUMMARY.

The paper attempts to give a synoptic and constructive account of our present day knowledge on the problem of prussic acid in grasses and urges that the investigations should be made in a more academic spirit.

A list of 88 grasses is compiled indicating the authors who have dealt with them and other points of interest.

The methods of extraction are discussed to some extent and the errors which may occur in quantitative determinations are pointed out.

In discussing the lethal dose the view is expressed that possibly another toxic substance besides prussic acid may be involved in the rapid death of animals. A hint is given how to verify that contention, the important point being to find out how much HCN is *liberated* in the animal and not how much is *introduced*.

The question of antidotes is only briefly referred to.

The discussion of the external conditions leading the plant to toxicity shows clearly how climate and soil interfere with the metabolism of the plant. Climate and soil, diurnal and seasonal variations and the effect of wilting are discussed.

The origin of prussic acid in the plant is still an unsolved problem. The most important views and theories of the past and present are reviewed and on that basis, the theory of Treub is given the benefit of a good working hypothesis.

Numerous experiments show the various effects of HCN on the plant and demonstrate what strong doses it is capable of standing.

A discussion is devoted to the fermentation of dhurrin.

In the chapter " prussic acid as an organic compound " the view is expressed that the second toxic substance referred to in the beginning may possibly be an isonitrile which is much more toxic than the nitrile form producing glycosids.

The author describes some of his own experiments on *Eustachys paspaloïdes* and *Sorghum verticilliflorum*.

HCl has an inhibiting effect on prussic acid production, so has marked alkalinity and also pepsin + HCl.

During hay production no prussic acid escapes although some may be transformed into other substances. The hay still contains a considerable amount of prussic acid.

Heating the grass to 59° C. (probable temperature of wilting) and 70° C. releases as much HCN as the chloroform test.

Extractions made with 95 per cent. alcohol are safe, no prussic acid escapes during the process. 42·5 per cent. alcohol is not safe, the acid escapes during extraction.

Elimination or adjunction of Fe, Mg and Ca makes no difference, but Al and Mn have an inhibitory effect.

Lead acetate precipitates the ferment with denaturation. Lead acetate partly also precipitates the glycoside; a fact to be taken into account in quantitative tests.

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