The interfering substance was not determined. Askew (1933) has already been quoted as stating that the plant material should be distilled in a good volume of water, otherwise decomposition products appear in the distillate and interfere with the silver nitrate. According to Furlong (1914) hydrolytic and distillation products sometimes interfere with the silver nitrate or iodine titration methods.

The author has mainly used the picrate test for qualitative work and the alkaline titration method for quantitative work. Although the former is non-specific, the author has not obtained any indication of erroneous results with the test when working on plant material. With the latter method, however, difficulties have occasionally been encountered, some of which are recorded in Table 12.

TABLE 12.

Abnormal Results Obtained by the Alkaline Titration Method.

Material.	HCN Content: Author's Method.	HCN Content: Alkaline Titration Method.	Remarks.
		Per Cent.	
Peach kernels.....	0·171 per cent.	0·2133	On titration with silver nitrate a brown colour gradually developed. End-point not sharp.
Acacia albida (pods).....	Trace.....	0·0176	Picrate test gave negative result. On titration with silver nitrate the solution became somewhat turbid and deep brown in colour. End-point very indefinite.
Acacia albida (pods).....	Trace.....	0·022	
Acacia giraffae (pods)....	0·0166 per cent	0·0183	On titration with silver nitrate the solution developed a faint brown colour.
Acacia giraffae (pods)....	0·0162 per cent.	0·0262	On titration with silver nitrate the solution became somewhat turbid and brown in colour. End-point not sharp.

Trace = less than 0·01 mg. of hydrocyanic acid.

The fact that a brown colour developed, that the solution became turbid, that the end-point was indefinite, as well as the fact that consistently higher results were obtained with the alkaline titration method, clearly indicates the presence of interfering substances in the distillate.

A large number of plant specimens, in which there was no evidence of the presence of interfering substances in the distillate, were analysed according to both methods so as to compare the results obtained by them.

Some of these analyses are recorded in Table 13.

TABLE 13.

Comparison of the Ferric Thiocyanate and Alkaline Titration Methods.

Plant.	HCN Content: Author's Method.	HCN Content: Alkaline Titration Method.
	Per Cent.	Per Cent.
Cynodon plectostachyum.....	0·0563	0·0579
Dimorphotheca Ecklonis.....	0·0215	0·0215
Cynodon plectostachyum.....	0·0225	0·0234

In view of the fact that unsatisfactory results may be obtained with the iodine and silver nitrate titration methods, the author decided to adopt the ferric thiocyanate method, as outlined above, for the quantitative estimation of hydrocyanic acid in plants.

In Table 14 is recorded the time required for the removal of hydrocyanic acid from plant material by aeration as conducted by the author. It will be seen that aeration for 2 hours sufficed to remove the most if not all of the hydrocyanic acid from the specimens of plant material analysed.

TABLE 14.

Time Required for the Liberation of Hydrocyanic Acid from Plant Material.

Material.	Weight of Specimen.	HCN Content Mg. per 100 Gm.	Time Required for Satisfactory Removal of HCN.
Peach kernels.....	15 gm.	23·5	1 hour.
Cynodon plectostachyum.....	30 gm.	15·6	1¼ hour.

III. SOURCES OF HYDROCYANIC ACID.

A. CYANIDES.

Effluents from gold mines may contain cyanide in solution and by contaminating natural streams cause hydrocyanic acid poisoning in animals utilising the stream as a source of drinking water.

Preparations of hydrocyanic acid are on the market for the destruction of vermin and careless use of such preparations may lead to accidental poisoning. Furthermore, preparations of hydrocyanic acid may be used maliciously to poison stock.

Careless use of hydrocyanic acid in the fumigation of houses, fruit trees, etc., for the destruction of pests may also cause accidental poisoning.

B. PLANTS.

Cyanogenetic plants form by far the most important source of hydrocyanic acid. Steyn (1934) gives a fairly complete list of the known cyanogenetic plants in the Union of South Africa which are of importance to stock. During the last few years the author has tested all plants, received at this Institute, for hydrocyanic acid. Table 15 contains the results of this survey.

In the case of those plants marked with an asterisk the quantitative determinations were made as follows: A number of specimens of the plant were stored in water at room temperature and determinations made every 24 hours. The highest value obtained in this way is given in the table. All the other quantitative determinations were made after having stored the plant in a solution of mercuric chloride for a sufficiently long interval.

It has been shown that the alkaline titration method may occasionally yield abnormal results. In such cases the distillate developed a brown colour and became turbid, the end-point being indefinite. Such abnormal results are not included in Table 15.

The moisture content of the plants was not determined so that the hydrocyanic acid content of the plants is given in mg. per 100 gm. of weight of the plant as it was received in the laboratory.

TABLE 15.

Plant.	State.	Stage.	Part of Plant.	HCN Content Mg. per 100 Gm.	Method.
Amarantus angustifolius..	Dry.....	Late seeding.	Whole plant.	Positive...	Guignard.
*Acacia giraffae**.....	Dry.....	Ripe.....	Pods.....	18·4 mg.	Alkaline titration.
Acacia giraffae.....	Dry.....	Ripe.....	Pods.....	16·4 mg.	Thiocyanate.
Dimorphotheca caulescens.	Fresh....	Flowering....	Whole plant.	Strongly positive	Guignard.
Cotyledon campanulata...	Fresh....	No flowers or fruit	Leaves.....	Strongly positive	Guignard.
*Jatropha capensis**.....	Wilted...	Pre-flowering.	Leaves.....	0·15 mg.	Thiocyanate.
Acanthospermum australe.	Fresh....	Early seeding	Whole plant.	Strongly positive	Guignard.
Datura stramonium.....	Fresh....	Seeding.....	Leaves.....	Positive...	Guignard.
Mollugo cerviana.....	Fresh....	Flowering....	Whole plant.	Strongly positive	Guignard.
Acanthospermum hispidum	Fresh....	Early seeding	Whole plant.	Strongly positive	Guignard.
Melica decumbens.....	Fresh....	Early seeding	Whole plant.	Strongly positive	Guignard.
Poinciana pulcherrima....	Fresh....	Unknown....	Leaves.....	Strongly positive	Guignard.
Cynodon plectostachyum, Star Mubende Strain	Fresh....	Seeding.....	Leaves.....	83·4 mg.	Alkaline titration.
Cynodon plectostachyum, Star Fort Portal Strain	Fresh....	Seeding.....	Leaves.....	104·1 mg.	Alkaline titration.
Cynodon plectostachyum, Star Kozinga Channel Strain	Fresh....	Seeding.....	Leaves.....	88·6 mg.	Alkaline titration.
Cynodon plectostachyum, Star Mount Elgon Strain	Fresh....	Seeding.....	Leaves.....	93·9 mg.	Alkaline titration.
Sorghum verticilliflorum...	Fresh....	Seeding.....	Leaves.....	66·0 mg.	Alkaline titration.
*Lotonis laxa**.....	Dry.....	Late seeding.	Whole plant.	120·5 mg.	Alkaline titration.
Medicago sativa.....	Fresh....	Bud stage...	Whole plant.	2·0 mg.	Thiocyanate.
Dimorphotheca Ecklonis...	Fresh....	Flowering....	Leaves.....	174·9 mg.	Thiocyanate.
Sorghum vulgare var. Esland	Fresh....	Seeding.....	Leaves.....	6·6 mg.	Thiocyanate.
Sorghum vulgare var. Esland	Fresh....	Unripe.....	Seed.....	0·7 mg.	Thiocyanate.
Sorghum vulgare var. Feterita 1937/173	Fresh....	Seeding.....	Leaves.....	8·4 mg.	Thiocyanate.
Sorghum vulgare var. Feterita 1937/173	Fresh....	Unripe.....	Seed.....	1·27 mg.	Thiocyanate.
Sorghum vulgare var. 37R9	Fresh....	Seeding.....	Leaves.....	0·3 mg.	Thiocyanate.
Sorghum vulgare var. 37R9	Fresh....	Unripe.....	Seed.....	0·81 mg.	Thiocyanate.
Sorghum vulgare var. Neesii 37/150	Fresh....	Seeding.....	Leaves.....	0·3 mg.	Thiocyanate.
Sorghum vulgare var. Neesii 37/150	Fresh....	Unripe.....	Seed.....	0·53 mg.	Thiocyanate.
Sorghum vulgare var. Stella Red	Fresh....	Seeding.....	Leaves.....	1·03 mg.	Thiocyanate.
Sorghum vulgare var. Stella Red	Fresh....	Unripe.....	Seed.....	1·57 mg.	Thiocyanate.

TABLE 15 (*continued*).

Plant.	State.	Stage.	Part of Plant.	HCN Content Mg. per 100 Gm.	Method.
Sorghum vulgare var. White Persia	Fresh....	Seeding.....	Leaves.....	0·34 mg.	Thiocyanate.
Sorghum vulgare var. 37R37	Fresh....	Seeding.....	Leaves.....	2·35 mg.	Thiocyanate.
Sorghum vulgare var. 37R37	Fresh....	Unripe.....	Seed.....	0·67 mg.	Thiocyanate.
Sorghum vulgare var. G.S. Manchu 37/187	Fresh....	Seeding.....	Leaves.....	1·52 mg.	Thiocyanate.
Sorghum vulgare var. G.S. Manchu 37/187	Fresh....	Unripe.....	Seed.....	2·4 mg.	Thiocyanate.
Sorghum vulgare var. 37R29	Fresh....	Seeding.....	Leaves.....	0·21 mg.	Thiocyanate.
Sorghum vulgare var. 37R29	Fresh....	Unripe.....	Seed.....	2·65 mg.	Thiocyanate.
Sorghum vulgare var. Taungs pink 37/10	Fresh....	Seeding.....	Leaves.....	4·78 mg.	Thiocyanate.
Sorghum vulgare var. Taungs pink 37/10	Fresh....	Unripe.....	Seed.....	1·4 mg.	Thiocyanate.
Sorghum vulgare var. Ntweka 37/48	Fresh....	Seeding.....	Leaves.....	7·2 mg.	Thiocyanate.
Sorghum vulgare var. Ntweka 37/48	Fresh....	Unripe.....	Seed.....	1·8 mg.	Thiocyanate.
Urginea macrocentra	Fresh....	No flowers or fruit	Bulbs.....	Slightly positive	Guignard.
Urginea macrocentra.....	Fresh....	No. flowers or fruit	Leaves.....	1·3 mg.	Thiocyanate.
Adenia digitata.....	Fresh....	No flowers or fruit	Bulbs.....	14·7 mg.	Alkaline titration.
Sorghum vulgare.....	Fresh....	Ripe.....	Seed.....	0·72 mg.	Thiocyanate.
Sorghum vulgare.....	Fresh....	Ripe.....	Seed.....	1·2 mg.	Thiocyanate.
Tagetes minuta.....	Fresh....	Pre-flowering.	Whole plant.	Negative...	Guignard.
Tagetes minuta.....	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Tagetes minuta.....	Fresh....	Post-flowering	Leaves.....	Negative...	Guignard.
Berkheyopsis echinus.....	Dry.....	Flowering....	Whole plant.	Negative...	Guignard.
Boschia foetida (noenie bos)	Fresh....	Flowering....	Leaves and flowers	Negative...	Guignard.
Dipcadi sp. aff. *D. viride*	Fresh....	Flowering....	Leaves.....	Negative...	Guignard.
Dipcadi sp. aff. *D. viride*	Fresh....	Flowering....	Bulbs.....	Negative...	Guignard.
Datura arborea.....	Dry.....	Flowering....	Leaves.....	Negative...	Guignard.
Epaltes alata.....	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Thesium lineatum (Vaal Storm)	Dry.....	No flowers or fruit	Whole plant.	Negative...	Guignard.
Cadaba juncea (Swart Storm)	Dry.....	Pre-flowering.	Whole plant.	Negative...	Guignard.
Zea mays (maize).....	Dry.....	Ripe.....	Seed.....	Negative...	Guignard.
Kedrostis nana	Fresh....	No flowers or fruit	Tuber.....	Negative...	Guignard.
Cryptolepis decidua.....	Dry.....	Late seeding.	Leaves.....	Negative...	Guignard.
Dolichos axillaris.....	Dry.....	Late seeding.	Whole plant.	Negative...	Guignard.
Euphorbia sp. aff. *E. pubescens*	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Salsola kali.....	Dry.....	Pre-flowering.	Whole plant.	Negative...	Guignard.
Castanospermum australe (Australian Chestnut Tree)	Dry.....	Seeding.....	Leaves.....	Negative...	Guignard.
Urginea Burkei (Transvaal Slangkop)	Dry.....	Post-seeding..	Bulbs.....	Negative...	Guignard.
Amarantus paniculatus...	Dry.....	Late seeding.	Leaves and seed	Negative...	Guignard.

TABLE 15 (*continued*).

Plant.	State.	Stage.	Part of Plant.	HCN Content Mg. per 100 Gm.	Method.
Kalanchoe paniculata.....	Fresh....	Early seeding	Leaves.....	Negative...	Guignard.
Kalanchoe paniculata.....	Fresh....	Late flowering	Flowers.....	Negative...	Guignard.
Canavalia ensiformis.....	Fresh....	Early seeding	Leaves.....	Negative...	Guignard.
Sarcostemma viminale....	Fresh....	Post-seeding..	Stems.....	Negative...	Guignard.
Indigofera patens.....	Dry.....	Late seeding.	Whole plant.	Negative...	Guignard.
Senecio sp. ab. Kraam-winkel, Settlers, July, 1937	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Cynanchum capense.....	Dry.....	No flowers or fruit	Whole plant.	Negative...	Guignard.
Zygophyllum morgsana...	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Galenia africana.....	Dry.....	Flowering....	Whole plant.	Negative...	Guignard.
Ochna pulchra	Dry.....	Flowering....	Leaves.....	Negative...	Guignard.
Synadenium arborescens..	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Tripteris aghillana.....	Dry.....	Flowering....	Whole plant.	Negative...	Guignard.
Chironia baccifera.....	Dry.....	Flowering....	Whole plant.	Negative...	Guignard.
Lotonis bainesii.....	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Crotolaria distans.....	Dry.....	Late seeding.	Whole plant.	Negative...	Guignard.
Melolobium microphyllum.	Fresh....	No flowers or fruit	Whole plant.	Negative...	Guignard.
Atriplex rosea.....	Fresh....	Flowering....	Whole plant.	Negative...	Guignard.
Cissus quinata.....	Fresh....	Seeding.....	Whole plant.	Negative...	Guignard.
Othonna cluytiafolia.....	Fresh....	Flowering....	Leaves and flowers	Negative...	Guignard.
Gliricidia maculata.....	Dry.....	No flowers or fruit	Leaves.....	Negative...	Guignard.

To the results in Table 15 should be added that Dr. D. G. Steyn of this Institute (private communication to the author) conducted a large number of tests on fresh and wilted specimens of *Tribulus terrestris*, barley and oats with negative results.

IV. FACTORS CONCERNED IN THE CAUSATION OF HYDROCYANIC ACID POISONING.

A. CONCERNING THE ANIMAL.

(1) *Quantity of the plant ingested.*

One of the earliest symptoms of hydrocyanic acid poisoning is listlessness, i.e., the animal stops feeding. It will depend on the quantity of the plant ingested by the time this stage is reached whether sufficient hydrocyanic acid has been ingested to cause fatal poisoning. Hungry or voracious animals will more likely ingest such a quantity of the plant. In the case of grazing animals the more prevalent the plant the greater is the danger of animals ingesting a lethal quantity of it.

(2) *The previous diet of the animal.*

Hydrocyanic acid is usually contained in the plant in the form of a cyanogenetic glucoside which is hydrolysed by an enzyme. This enzyme

may be present in other plants, e.g., emulsin is contained in many plants (Wehmer, 1929) and, if such plants were present in the diet, an excess of the enzyme will be present in the stomach facilitating the rapid liberation of hydrocyanic acid.

Steyn (1931) found sulphur to be an excellent preventive of hydrocyanic acid poisoning and sulphur is now extensively fed for this purpose. It is a well-known fact that glucose and other carbohydrates form innocuous cyanhydrin with hydrocyanic acid and the presence of these substances in the diet will consequently protect the animal to a greater or lesser extent against hydrocyanic acid. Couch (1932) states that lucerne hay and linseed cake retard the production of hydrocyanic acid and thus act as preventives. Keeser (1930) found that rabbits on a diet of green feed and ferrous chloride showed a greater resistance to cyanide poisoning as compared to rabbits on milk, rice or green feed only, and ascribes the increased resistance of the former rabbits to the increased iron content of the tissues. Some plants contain relatively large quantities of nitrates, e.g., *Tribulus terrestris* (Rimington and Quin, 1933). These authors found that from the nitrate nitrite is formed in the animal under the influence of an enzymic oxidation-reduction system. Nitrite is a well-known remedy for hydrocyanic acid poisoning and such plants may, therefore, act as preventives. Furthermore, nitrate has on a number of occasions been encountered in appreciable quantities in water from boreholes or wells in the Union of South Africa.

(3) *pH of the stomach contents.*

The activity of enzymes is greatly influenced by the pH of the medium. Furthermore, should the stomach contents be alkaline, the hydrocyanic acid liberated will be bound in the form of cyanides which are absorbed less rapidly than hydrocyanic acid.

In this respect it must be pointed out that farmers in the Union of South Africa often add lime to the diet of their stock and, furthermore, that lime often occurs in appreciable quantities in natural waters. The action of the lime will be to cause a shift in the pH of the ruminal contents to the alkaline side and to precipitate hydrocyanic acid in the form of calcium cyanide.

B. Concerning the Plant.

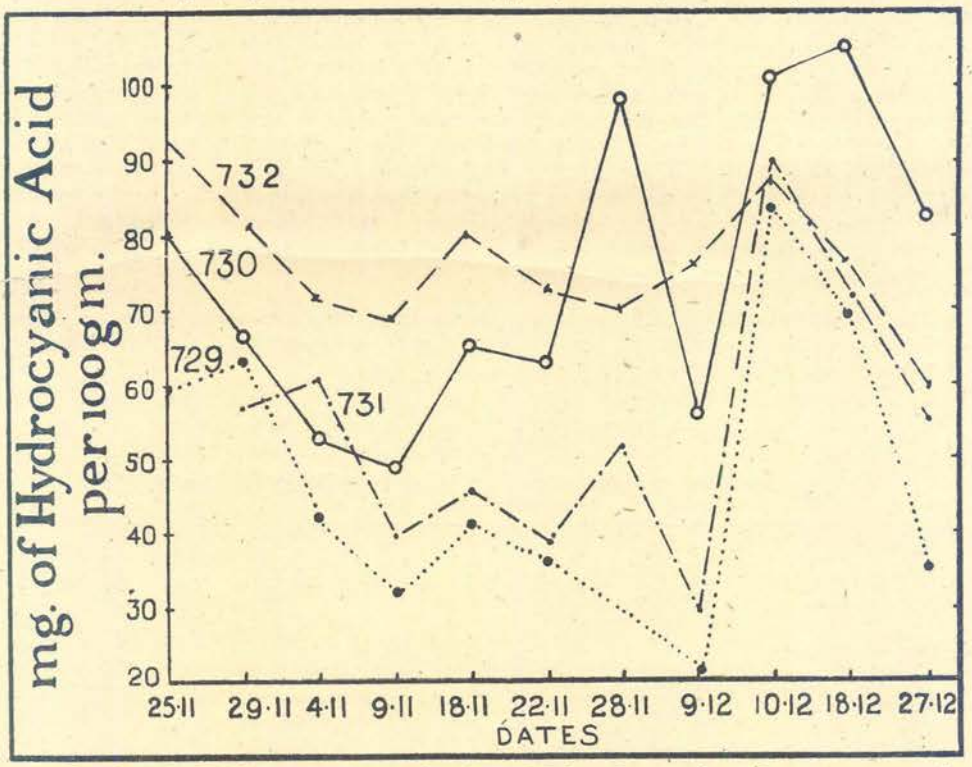
(1) *The hydrocyanic acid content of the plant.*

This is obviously of the greatest importance. Very many plants are cyanogenetic but all are definitely not dangerous, since many contain only small quantities of hydrocyanic acid. Hydrocyanic acid in plant material in the form of cyanogenetic glucosides is obviously not as dangerous as an equal quantity in aqueous solution since time is required to liberate the hydrocyanic acid from the glucoside. This interval enables the animal by the rapid elimination of hydrocyanic acid, especially through the exhaled air, to rid itself of fair amounts of the poison.

Graph I represents the hydrocyanic acid content of four strains of *Cynodon plectostachyum* (giant star grass) and Graph II represents the hydrocyanic acid content of *Dimorphotheca Ecklonis* (bietou).

GRAPH I.

Hydrocyanic acid content of Cynodon plectostachyum.



In Graph I 729 represents the Star Mubende strain, 730 represents the Star Fort Portal strain, 731 represents the Star Kozinga Channel strain and 732 the Star Mount Elgon strain.

TABLE 16.

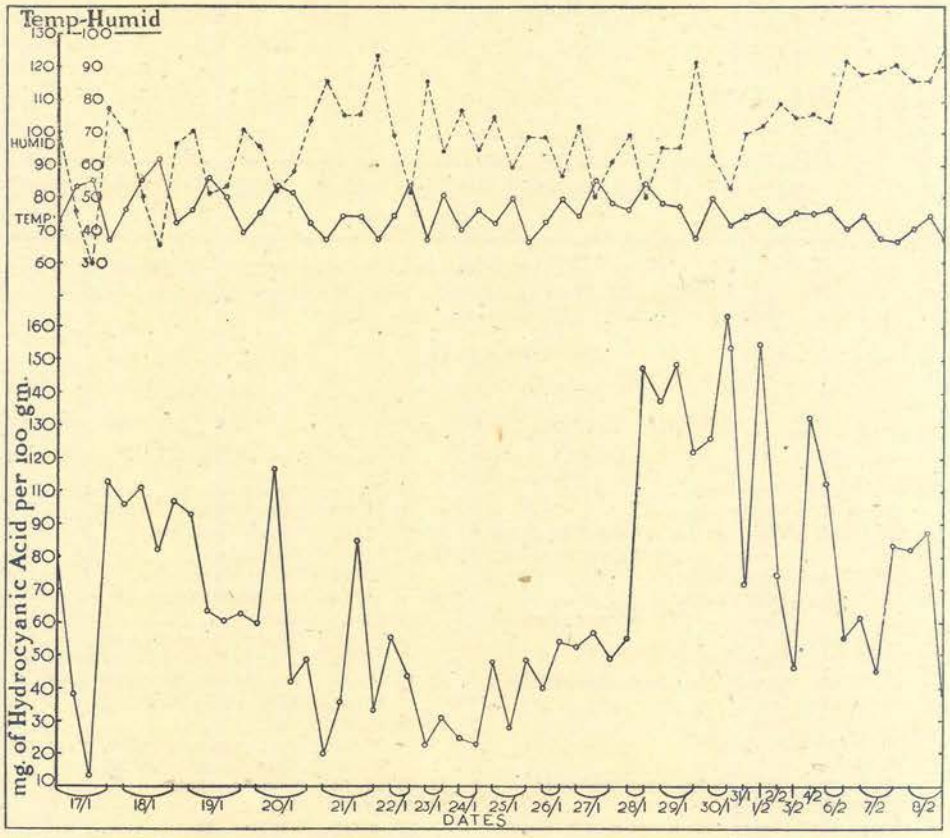
Date.	Weather Conditions.	Date.	Weather Conditions.
21–24/10/40	Very warm.	21/11/40	Cloudy, warm, a little rain.
25/10/40	Very warm.	22/11/40	Cloudy, warm, a little rain.
28/10/40	Very warm, cloudy in afternoon, and rain from 7–8 p.m.	23–24/11/40	Cloudy and cooler.
		25–27/11/40	Warmer.
29/10/40	Cool, cloudy.	28/11/40	Fairly warm.
30– 1/11/40	Very warm.	6– 8/12/40	Cloudy, warm.
2– 3/11/40	Cloudy, cool, large amount of rain	9/12/40	Warmer.
4/11/40	Cloudy, cool.	10/12/40	Extremely warm, grass wilted.
5– 7/11/40	Very warm.	11/12/40	Cloudy, rain towards evening.
8/11/40	Cloudy, cool, large amount of rain	12–13/12/40	Slightly cloudy, fairly warm.
9/11/40	Cool.	14/12/40	Rain, fairly cool.
10–13/11/40	Warm.	15–16/12/40	Fairly warm.
14–15/11/40	Fairly warm.	17/12/40	Cool, raining all day.
16–17/11/40	Very warm.	18/12/40	Warmer, sun shining.
18/11/40	Very warm, thunderclouds.	22–23/12/40	Raining, cool.
19/11/40	Warm.	24–26/12/40	Warm.
20/11/40	Warm, thunder clouds during night.	27/ 2/41	Raining, cool.

The material for these determinations was collected from plots of the plants in the Onderstepoort Poisonous Plant Garden. The leaves of the plants were utilised. Immediately after collection the material was taken to the laboratory, rapidly minced and suitable quantities placed in flasks containing 200 c.c. of water and 20 c.c. of a 5 per cent. mercuric chloride solution. The flasks were sealed and stored for 1 week. The specimens were steam distilled after the addition of stannous chloride and tartaric acid, and the distillate collected in a flask containing 10 c.c. of a 10 per cent. solution of sodium hydroxide. The hydrocyanic acid was determined by the alkaline titration method. It had been determined that the quantity of hydrocyanic acid, liberated during the week's storage, very closely approached the maximum hydrocyanic acid content of the plant specimens, so that storage for longer periods was unnecessary.

Both Graphs I and II serve to demonstrate the great variability of the hydrocyanic acid content of the plants. In connection with this variability the following should be borne in mind.

GRAPH II.

Hydrocyanic acid content of Dimorphotheca Ecklonis.



(*a*) *Soil.*—The character and composition of soil influences the hydrocyanic acid content of plants. Thus Steyn (1934), could detect hydrocyanic acid in maize only when grown on red sandy soil and not when grown on black clay soil. Busso (1934) states that heavy fertilizing with nitrates favours a high hydrocyanic acid content. According to Hadley (1938), a high level of available nitrogen and a low level of available phosphorus increases the hydrocyanic acid content whereas a low level of available nitrogen and a high level of available phosphorus decreases the hydrocyanic acid content of plants. Vinall (1921) states that plants grown on poor soil contain less hydrocyanic acid, especially if the soil is low in nitrates, and that on the addition of nitrates the hydrocyanic acid content is increased.

Willaman and West (1915) state that when sorghum is grown on poor, unfertile soil added nitrate may slightly increase the amount of hydrocyanic acid in the plant but that with a fertile soil and abundant nitrogen this effect may not be produced. They add that climate and variety may be more important factors than soil nitrogen in determining the amount of hydrocyanic acid in the plant.

(*b*) *Climate.*—It is a well-known fact that climatic conditions greatly influence the hydrocyanic acid content of plants. Ivanow and Smirnova (1935) state that *Sorghum vulgare* and *Andropogon sorghum* grown in darkness show a higher hydrocyanic acid content than when grown in the light. Treub (Robinson, 1930) found that the darkening of the plant *Pangium edule* causes a decrease in its hydrocyanic acid content. Doak (1933) states that clovers show day to day and seasonal variations in their hydrocyanic acid content and subsequently (Doak, 1936) mentions hourly variation in the hydrocyanic acid content of clover.

Narashima Acharya (Leeman, 1935) found in *Sorghum vulgare* an increase of hydrocyanic acid production from early morning to 2 p.m., after which there is a slight decline to 6 p.m., followed by a rapid decline during the night.

According to Hadley (1938) drought is important since it keeps the plants short when the hydrocyanic acid content is higher. Gusev and Vertela (1936) state that sorghum crops may be toxic to stock in one year but not in the next. Howes (1933) states that (1) the season of the year, and (2) the area where grown are two important factors influencing the hydrocyanic acid content. According to Meadly (1934) Sudan grass is most likely to be dangerous when stunted by frost or dry conditions. Henrici (1926) demonstrated that certain grasses which do not show any hydrocyanic acid in the normal fresh state develop large quantities of the acid under conditions of wilting.

Thus Steyn (1934) sometimes found hydrocyanic acid in fresh specimens of various *Cynodon* spp., whereas in the wilted state large amounts of hydrocyanic acid were always present. Steyn (1934) also mentions that frost may cause an increased hydrocyanic acid content of cyanogenetic plants. Willaman and West (1916) found that with an adequate water supply the hydrocyanic acid content of sorghum was low, whereas an inadequate water supply led to a high hydrocyanic acid content.

Considering Graph I in conjunction with the data on the climatic conditions in Table 16 it is evident that under warm, dry conditions the hydrocyanic acid content of the grasses increases and decreases under cooler, humid

conditions. Graph II demonstrates this even better. Graph I clearly shows the tremendous increase in the hydrocyanic acid content under conditions of wilting.

(*c*) *Age of plant.*—Ivanow and Smirnova (1935), Gusev and Vertela (1936), Coleman (1934), Tarantino (1935), Vinall (1921), Swanson (1921), and Hadley (1938) all state that the hydrocyanic acid content of plants (*Sorghum* spp.) decreases with age.

Willaman and West (1915) state that during the first three or four weeks of the plant's (sorghum) life the hydrocyanic acid is concentrated in the stalks and that it then rapidly decreases and disappears there, but apparently persists in the leaves in decreasing percentages till maturity.

(*d*) *Variety.*—Willaman and West (1916) found varietal differences a larger factor than conditions of growth in determining the amount of hydrocyanic acid in sorghum plants. According to Doak (1933), the hydrocyanic acid content is correlated with the strain of clover.

Graph I clearly shows the difference in the hydrocyanic acid content of four varieties of *Cynodon plectostachyum* (GIANT STAR GRASS). From Table 15 it is evident that strains of *Sorghum vulgare* vary greatly in their hydrocyanic acid content.

(*e*) *Individual variations.*—Doak (1933) states that individuals of one and the same strain of clover vary in their hydrocyanic acid content. The author has made similar observations with a number of plants.

(*f*) *Part of plant.*—Juliano and Guerrero (1935) tested the fruit, seeds flowers, stems, roots, leaves, and bark of stems and roots of a number of plants and found that hydrocyanic acid may be present in one or more of the above and not in the others, or may be present in all and that the hydrocyanic acid content of any one part may be greater than that of any other part. Martin, Couch and Briese (1938) determined the distribution of hydrocyanic acid in the heads, peduncles, sheaths, individual leaves and internodes of sorghum plants aged 2 months or more and grown in different areas. They found the hydrocyanic acid content of the leaves to be 3-25 times that of the corresponding stalks of plants which had reached the "boot" stage. The heads and leaf sheaths were found by them to be low in hydrocyanic acid. These authors also found that the younger leaves contained more hydrocyanic acid than the older leaves and that the axillary branches contained more hydrocyanic acid than the older main stalks. It is thus obvious that different parts of cyanogenetic plants may vary greatly in the hydrocyanic acid content, the most hydrocyanic acid occurring in those parts in which the processes of metabolism are most active.

(*g*) *State of the plant.*—According to the work of Dowell (1919) the quantity of hydrocyanic acid set free from *Andropogon sorghum* during drying depends on the speed of drying. Thus, if rapidly dried less hydrocyanic acid is set free than when slowly dried. According to Swanson (1921) Sudan grass dried in an oven loses a little hydrocyanic acid, whilst more hydrocyanic acid is lost if dried in the sun. Dried in the shade no hydrocyanic acid or only a trace remains.

Vinall (1921) states that the Australians contend that sorghum plants attacked by insects are more poisonous than normal plants. Willaman and West (1915) state that Balfour noticed that plants infected with *Aphis sorghi* contained more hydrocyanic acid than uninfected plants. Steyn (1934) states that bruising and trampling may cause an increase of hydrocyanic acid in cyanogenetic plants.

The fact that the hydrocyanic acid content of cyanogenetic plants is subject to great variation is extremely important since it clearly demonstrates that the danger of a cyanogenetic plant to stock should not be assessed on any one analysis of the plant, or even on the average hydrocyanic acid content of the plant. On the contrary, the danger of such a plant to stock should be judged on the maximum amount of hydrocyanic acid it may develop. Otherwise, a cyanogenetic plant may be regarded as safe and used for grazing when for a time no harm may result until conditions occur leading to the development of the maximum hydrocyanic acid content with fatal results to the animals consuming the plant.

In the present state of our knowledge no reliable criterion exists as to what hydrocyanic acid content of a plant should be regarded as dangerous to stock. Seddon and King (1930) regard a hydrocyanic acid content of 0·05 per cent. of the dry plant and 0·02 per cent. of the fresh plant as dangerous to stock. The following criticism can, however, be levelled at their work:—

(i) The plant material was ground or minced reducing it to a far finer state of division than would be the case by mastication, especially since ruminants, when grazing masticate very imperfectly. The finer the state of division of the plant material, the more rapid the liberation of hydrocyanic acid will be.

(ii) The plant material was drenched so that all the material entered the rumen at the same time instead of gradually, as would be the case with normal feeding. Thus with normal feeding more time is allowed, so that the animal can rid itself of quantities of the poison by excretion.

(iii) Enzyme was also administered so that an excess was present in the rumen which need not naturally be the case.

According to the above objections it is evident that in many cases a hydrocyanic acid content above that given by Seddon and King (1930) may not prove fatal. The level established by Seddon and King (1930) would, therefore, appear to be such that no plant containing less hydrocyanic acid will be fatal, whereas plants containing more hydrocyanic acid may or may not cause fatal poisoning, depending on all the factors enumerated in the preceding pages.

(2) In Table 17 are given the free and total hydrocyanic acid contents of a few plants.

TABLE 17.

Free and Total Hydrocyanic Acid contained in Various Plants.

Plant.	Free HCN Mg. per 100 Gram.	HCN Content: Mg. per 100 Gram.	Per Cent. Free HCN.
Cynodon plectostachyum.....	18·7	26·8	70
Dimorphotheca Ecklonis.....	21·5	89·7	24
Cynodon plectostachyum.....	23·1	24·1	96
Cynodon plectostachyum.....	20·3	25·6	79

As shown previously the free hydrocyanic acid was determined by placing the plant material, minced, immediately after collection of the specimen, in boiling water containing mercuric chloride. The enzyme is thus destroyed and only the hydrocyanic acid present in the free state in the plant determined. The total hydrocyanic acid was determined after storing the plant for a week in an aqueous solution of mercuric chloride, so as to allow the liberation of all the hydrocyanic acid bound in glucoside form. Hydrocyanic acid in the free state is obviously more dangerous than that in a conjugated form and this factor should be observed in assessing the danger of a cyanogenetic plant.

(3) *The quantity of enzyme in the plant.*

A cyanogenetic plant may be deficient in the enzyme responsible for the hydrolysis of the cyanogenetic glucoside. Finnemore (1931) found this to be the case with *Eremophila maculata*. Using the picrate test the author has encountered plants which give a slightly positive test but on the addition of emulsin give a strongly positive test. This factor should also be borne in mind when assessing the danger of a cyanogenetic plant to stock but it must be remembered, as pointed out previously, that the necessary enzyme may be present in other plants included in the diet of the animal. Thus Finnemore (1931) found the enzyme, deficient in *Eremophila maculata*, to be present in *Acacia Georgina*. Seddon and King (1930) found that sheep on certain natural pastures may gather sufficient enzyme to split a minimal fatal dose of a cyanogenetic plant which in itself contains practically no enzyme.

At this stage it will be of interest to consider what rôle is played by hydrocyanic acid in the metabolism of the plant.

According to Willaman and West (1916) the following uses have been attributed to glucosides in plants:—

(i) A protective agent against bacteria and other enemies by means of the poison set free when some glucosides are hydrolised.

(ii) A reserve food material in the plant.

(iii) An inactive form of stimulating hormone set free when necessary by a glucosidase.

(iv) A harmless compound absorbing injurious products of metabolism.

(v) An inactive storage of respiratory pigments.

(vi) A necessary intermediate product of protein metabolism.

These authors add that hydrocyanic acid, as such, is probably rather transitory in the plant and seldom occurs free in any appreciable amount. They state that the theory of food storage is not supported by the fact that there is no consistent daily variation in the amount of dhurrin in the plant. Willaman and West (1916) find the hormone theory most acceptable on the following grounds:—

(i) With an adequate water supply the hydrocyanic acid content of the plant is low, whereas with a decreasing water supply it increases. Thus with sufficient water, other factors being equal, the means of the plant for growth is adequate so that the plant needs less glucoside. With a decreasing water supply the plant may need the hormone stimulus for growth and thus more glucoside is produced.

(ii) Poor conditions of growth were associated with a high hydrocyanic acid content. This fact also supports the theory that the glucoside may be a harmless compound absorbing injurious products of metabolism.

On a previous occasion Willaman and West (1915) had stated that the cyanogenetic glucoside is evidently related to the vital processes of the plant as it occurs in the largest quantity in those parts of the plant which are most active photosynthetically and during those stages when the plant is developing most rapidly. Swanson (1921) also states that in Sudan grass hydrocyanic acid is present in the greatest quantity in the parts of greatest vegetative activity, thus supporting the theory that hydrocyanic acid is an intermediate product between nitrates and amino-acids.

Bach (Dunstan and Henry, 1906) extends the theory of Meyer and Schulze and suggests that from the small amounts of nitrate in the cell-sap, small amounts of nitric acid are formed by the large quantities of oxalic and carbonic acid usually present. The free nitric acid is continually reduced by formaldehyde producing hydroxylamine which immediately produces formaldoxime with further formaldehyde. The formaldoxime may be converted into isomeric formamide which on dehydration would yield hydrocyanic acid and water or the formamide may be hydrolysed yielding ammonium formate, thus supplying ammonia and formic acid. Dunstan and Henry (1906) remark as follows on the theory of Bach:—

(i) A fundamental objection to the theory is the fact that hydroxylamine is poisonous to protoplasm, but this can be explained away by accepting that the hydroxylamine is immediately transformed into a stable and innocuous oxime.

(ii) Physiological support of the theory is found in the fact that the supposition that nitric acid is reduced by formaldehyde agrees with the fact that whilst nitrates occur abundantly in stem structures of green plants they are absent or present in only small amounts in the leaves, i.e., in the organs supposed to be most active in the production of formaldehyde. On other grounds many workers have stated that the reduction of nitrates is most active in the leaves. There can be no objections to the reduction of nitric acid as follows, though no proof for such a process exists:—

$$NO_3H \rightarrow NO_2H \rightarrow NH_2OH \text{ (hydroxylamine)}.$$

(iii) The isomeric change of formaldoxime into formamide is probable since Dunstan and Bossi have shown that formaldoxime yields ammonia and formic acid when boiled with dilute hydrochloric acid.

(iv) Furthermore, Scholl reports that formamide on dehydration forms hydrocyanic acid.

(v) The hydrolysis of formamide to formic acid and ammonia is a familiar chemical reaction.

The theory of Bach is supported by the fact that wilting causes an increase in the hydrocyanic acid content of plants. Dunstan and Henry (1906) state in conclusion that Bach thus offers no suggestion that hydrocyanic acid takes any further part in the metabolic process and that he apparently regards it as an accidental product of little importance.

The consensus of opinion, however, appears to be in support of the theory that hydrocyanic acid is an intermediate stage in protein metabolism. Pfluger (Dunstan and Henry, 1906) advances the hypothesis that in ordinary proteids the nitrogen is in the form of amino-groups, whilst in living protoplasm it is in the form of cyanogen radicles. Latham (Dunstan and Henry, 1906) also assigns an important rôle to the cyanogen radicle and to hydrocyanic acid in the constitution and natural synthesis of animal proteids.

According to Dunstan and Henry (1906) evidence in favour of the view that hydrocyanic acid plays a rôle in protein metabolism has been accumulated mainly in 3 ways:—

(i) By physiological experiments.

(ii) By the investigation of the distribution of cyanogenetic compounds in the vegetable kingdom.

(iii) By chemical investigation of the process of cyanogenesis in plants.

Thus in cases where there is little or no cyanogenetic glucosides in seeds there is on germination a large and rapid increase in the total amount of hydrocyanic acid available. This increase reaches a maximum after which a decrease occurs in some cases to zero. Furthermore, manuring of maize and sorghum increases the hydrocyanic acid content as does potassium nitrate in the case of *Phaseolus lunatus*. Treub (Dunstan and Henry, 1906) is of the opinion that hydrocyanic acid plays an important part in plant metabolism and that hydrocyanic acid may be a step in the transformation of the " inorganic " nitrogen of nitrates to the " organic " nitrogen of proteids.

Gautier (Dunstan and Henry, 1906) supposes that the free nitric acid of the cell sap reacts with formaldehyde forming hydrocyanic acid, carbon di-oxide and water and that the hydrocyanic acid enters into chains with formaldehyde ultimately forming the unit of the proteid molecule. According to Leeman (1935), Menaul obtained hydrocyanic acid by adding 2 c.c. of 40 per cent. formaldehyde and 1 gm. of potassium nitrate to 400 c.c. of water saturated with carbon di-oxide. The solution was acidified. Menaul, therefore, rightly concludes that hydrocyanic acid may be formed in plants by the action of formaldehyde on nitrates.

Hebert (Dunstan and Henry, 1906) demonstrated that in *Aquilegia vulgaris* hydrocyanic acid occurs only in the parts containing chlorophyll and concludes that the formation of hydrocyanic acid is dependent on the formation of formaldehyde. This would serve to support the theory of Gautier. Treub (Dunstan and Henry, 1906), however, has shown that the supply of sugar influences the production of hydrocyanic acid more than light and, therefore, modifies the theory of Gautier by regarding that the reduction of the nitric acid of the cell sap is due to sugar.

The following is quoted from an article by Greshof (1906):—If it is accepted that hydrocyanic acid plays a rôle in proteid synthesis the fact that some plants do not contain hydrocyanic acid can be explained by assuming that in such cases the hydrocyanic acid is so rapidly transformed that it cannot be detected. It is also possible that hydrocyanic acid in all plants may not necessarily be a stage in the proteid synthesis but arises in other ways. Hydrocyanic acid occurs mainly in two combinations in plants, namely with acetone as acetonecyanohydrin, and with benzaldehyde as benzaldehydecyanohydrin. On chemical grounds the acetonecyanohydrin may be regarded

as primary material for proteid synthesis. The benzaldehydecyanohydrin is a stable compound and it is not clear in what way this compound could act as primary material in proteid synthesis. Since the proteid molecule contains aromatic groups the step from benzaldehyde-cyanohydrin to tyrosin is, however, not unthinkable.

Leeman (1935) disagrees with the theory of Gautier on the grounds that too precise an idea is advanced of the rôle played by hydrocyanic acid in protein synthesis, especially since the supposed reactions involved are purely inventions based on scanty facts. On the other hand Leeman regards the theory of Treub as much more likely of being of some value because it is held in general terms and does not attempt to describe the details of the process. Leeman discusses the objections to, and the facts in favour of, the the theory of Treub including the work of Rosenthaler, Ravenna and Zamorani, Stekelenburg, and Ravenna, and states that taking into account all facts he feels inclined to grant the hypothesis of Treub the title of a good working hypothesis. Finally Leeman points out that this does not wholly do away with the possibility of creating the hydrocyanic acid by other means such as those described by the theory of Bach or shown by the experiments of Plummer who obtained hydrocyanic acid by oxidation of albumins.

V. THE HYDROCYANIC ACID CONTENT OF THE ORGANS OF NORMAL ANIMALS AND ANIMALS POISONED BY HYDROCYANIC ACID.

A. Introduction.

The presence of a poison in the organs of an animal does not necessarily prove that the animal died from poisoning by that substance since a sublethal dose may have been ingested. The quantity of poison taken will reflect itself in the quantity of the poison present in the organs of the animal. It may thus be possible to judge by the quantity of the poison present in an organ whether an animal has been fatally poisoned by that substance.

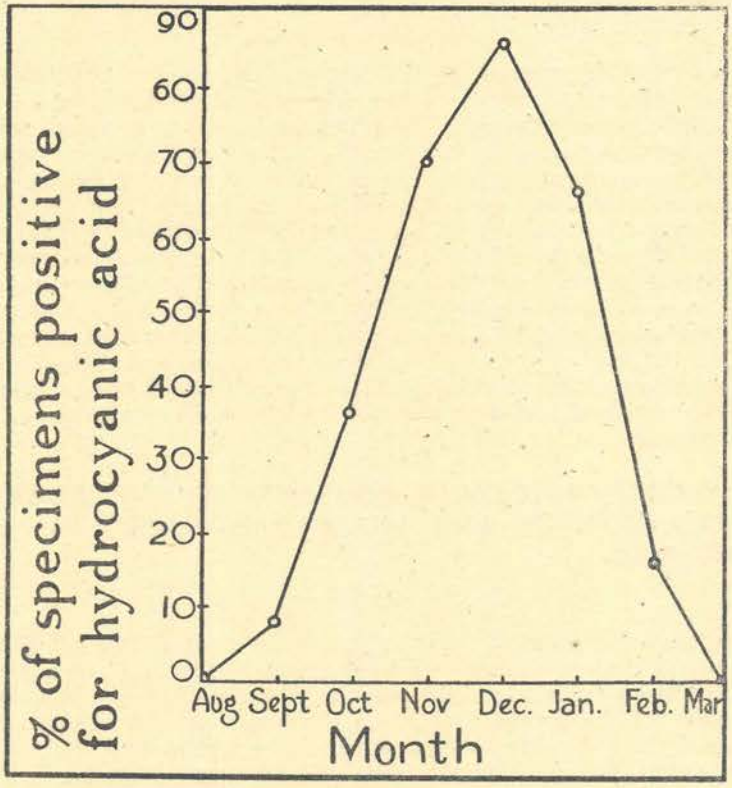
Analyses of all specimens of ruminal contents received at this Institute for analysis for a period of approximately two years revealed that hydrocyanic acid, in relatively large quantities, was present in many cases in which death was found to be due to some other cause. A total of 361 specimens was analysed, of which 88 or 24 per cent. were found to be positive for hydrocyanic acid.

In Graph III are represented the percentage of specimens, positive for hydrocyanic acid, during the period August, 1938 to March, 1939. The total number of specimens analysed during each month, is as follows:— August 8, September 12, October 10, November 31, December 31, January 33, February 13, March 14.

It is evident that hydrocyanic acid was found most frequently during the summer months in the ruminal contents of animals, which had died from causes other than hydrocyanic acid poisoning. It is, therefore, obvious that the presence merely of hydrocyanic acid in the organs of an animal is of no significance. The frequent occurrence of hydrocyanic acid in stomach contents of animals is not at all surprising in view of the prevalence of cyanogenetic plants in the Union of South Africa.

GRAPH III.

Percentage of routine specimens which contain hydrocyanic acid.



Since this Institute is frequently called on to diagnose the possibility of hydrocyanic acid poisoning, as a cause of death, on the analyses of organs, it was decided to conduct these investigations.

B. THE HYDROCYANIC ACID CONTENT OF THE ORGANS OF NORMAL ANIMALS.

Waller (1910) failed to detect hydrocyanic acid in the distillates of various organs of humans and cats which had not received hydrocyanic acid. Gettler and Baine (1938) also failed to detect hydrocyanic acid in various organs of humans not poisoned by hydrocyanic acid and state that 0·01 mg. of hydrocyanic acid can readily be detected by their method.

Ganassini (Autenreith, 1928) found hydrocyanic acid in the distillates of organs of healthy animals although he is positive that the hydrocyanic acid was not originally present in the organs. He, therefore, considers that traces of hydrocyanic acid are formed from protein substances and xanthine bases at high temperatures due to overheating. Thus hydrocyanic acid was formed from haematin at 200°. This contention is supported by the fact that Plimmer (Autenrieth, 1928) has demonstrated that hydrocyanic acid is formed from protein substances by oxidation. Klein (1932) has already been quoted as stating that traces of hydrocyanic acid may develop by direct

heating of aqueous extracts of plants containing acids, sugars and nitrites or nitrates. Autenrieth (1928), however, with direct heating never obtained hydrocyanic acid from organs and blood unless hydrocyanic acid was originally present.

Since in the method, adopted by the author, a water bath is used for heating purposes the fact that hydrocyanic acid may result from overheating (direct heating) is of no significance.

It is also of interest to note that, whereas Witthaus (1911) states that thiocyanates may yield free hydrocyanic acid on distillation with a mineral acid, Ganassini (Autenrieth, 1928) disagrees. The latter author distilled organic substances in the presence of potassium thiocyanate with tartaric or sulphuric acid and obtained no hydrocyanic acid but traces of free thiocyanic acid if large quantities of potassium thiocyanate were present. It is, therefore, unlikely that any interference in the author's method will result from this source.

In this work the author has not employed the organs of normal animals but those of animals which died from causes other than hydrocyanic acid poisoning and had been on a diet of lucerne hay, maize silage, crushed maize and veld hay.

TABLE 18.

*The hydrocyanic content of the liver and ruminal contents of sheep not poisoned by hydrocyanic acid and the development of hydrocyanic acid during decomposition.**

Sheep.	Material.	Weight.	Days of Decomposition.	Mg. HCN per 100 gm.	Cause of Death.
		Gram.			
59763	Ruminal contents.....	100	0	0·008	*Cadaba juncea* poisoning.
	Ruminal contents.....	100	2	0·004	—
	Ruminal contents.....	100	5	0·005	—
	Ruminal contents.....	100	7	0·005	—
	Liver.....	100	0	0·001	—
	Liver.....	100	2	0·002	—
	Liver.....	100	5	0·001	—
	Liver.....	100	7	0·004	—
65177	Ruminal contents.....	100	0	0·003	Enzootic icterus.
	Ruminal contents.....	100	3	0·003	—
	Ruminal contents.....	100	7	0·005	—
	Liver.....	100	0	0·003	—
	Liver.....	100	3	0·005	—
	Liver.....	150	7	0·005	—
64151	Ruminal contents.....	100	0	0·002	Enzootic icterus.
	Ruminal contents.....	100	3	0·004	—
	Ruminal contents.....	100	7	0·002	—
	Liver.....	50	0	0·001	—
	Liver.....	60	3	0·002	—
	Liver.....	60	7	0·001	—

* These, and all subsequent analyses of the organs of animals, were done according to the ferric thiocyanate method as modified by the author.

The results are contained in Table 18. It will be seen that traces of hydrocyanic acid were found both in the ruminal contents and liver. In this respect it should be pointed out that traces of hydrocyanic acid have

frequently been found in plants considered to be non-cyanogenetic, e.g., lucerne, and this undoubtedly explains the presence of hydrocyanic acid in the ruminal contents. The hydrocyanic acid in the liver may arise from any of the following sources: (1) it may be obtained from the rumen; (2) it may be a normal constituent of the liver; or (3) it may even have been formed during the analysis. The quantities of hydrocyanic acid found are, however, so small as not to be of the slightest significance. In view of this fact, the author made no attempt to determine the actual source of the hydrocyanic acid in the liver.

It has been shown that under field conditions hydrocyanic acid is frequently demonstrated in appreciable quantities in the ruminal contents and consequently larger quantities of hydrocyanic acid will probably be found in the livers of animals dying from causes other than hydrocyanic acid poisoning.

C. Is Hydrocyanic Acid formed during the Decomposition of Normal Organs?

McNally (1937) states that traces of hydrocyanic acid are produced during the first few days of putrefaction. Gettler and Baine (1938), working on brain and liver containing no hydrocyanic acid, found that hydrocyanic acid was formed during decomposition, the quantity formed increasing during the first seven days of decomposition and then decreasing. The largest quantity found was 0·031 mg. of hydrocyanic acid per 100 gm.

From Table 18 it is evident that the results obtained by the author, do not correspond with those of Gettler and Baine (1938), since if the formation of hydrocyanic acid, during decomposition under the experimental conditions of the author, did occur the quantities formed were such as to be of no significance.

The author has also analysed a number of specimens of liver which were submitted by farmers and arrived at this Institute in various stages of decomposition. In no case could hydrocyanic acid be demonstrated in quantities exceeding 0·005 mg. of hydrocyanic acid per 100 gm.

In this connection the work of Mochtar and van Veen (1941) is of great importance. These authors found *Pseudomonas*, *Citrobacter*, *Proteus* and *Alkaligenes* organisms in the blood of a seven day-old corpse and state that all these organisms, and especially the firstnamed, produced hydrocyanic acid when grown in fresh sheep, ox or human blood.

The above may account for the discrepancy between the results of the author and those of Gettler and Baine (1938), namely, the organisms may have been present in the organs used by Gettler and Baine (1938) and absent in those used by the author. It would, therefore, appear from the work of the author that hydrocyanic acid does not result from putrefactive changes but from certain organisms which may or may not be present during putrefaction.

D. The Hydrocyanic Acid Content of the Organs of Animals Poisoned by Hydrocyanic Acid.

Since the hydrocyanic acid content of the organs depends on the quantity of this poison ingested, these investigations were made in order to establish a level of hydrocyanic acid in an organ in the fresh state in such a way that

if a hydrocyanic acid content above this level was found, it would prove that a lethal quantity of hydrocyanic acid was ingested and if a hydrocyanic acid content below this level was found, it would prove that a sub-lethal quantity of the poison was ingested and that the animal had died from another cause. In hydrocyanic poisoning under field conditions (" Geilsiekte ") the history is usually that of sudden death, i.e., the owner finds the animal dead without having noticed it ill. Since a host of causes may be responsible for sudden death the above procedure would greatly assist in arriving at a definite diagnosis.

It is definitely not sufficient to analyse only the stomach contents since hydrocyanic acid has frequently been found in the ruminal contents of animals which had not died of hydrocyanic acid poisoning. Furthermore, the presence of hydrocyanic acid in the ruminal contents does not in any way prove that a lethal quantity of hydrocyanic acid has been absorbed. The main cause of hydrocyanic poisoning being cyanogenetic plants, it should also be remembered that the hydrocyanic acid present in the ruminal contents may for the greater part have been liberated after death. It is, therefore, essential to analyse also an organ to which the hydrocyanic acid must be conveyed via the blood.

In selecting such an organ the following should be borne in mind:—

(1) Since, within limits, the greater the quantity of hydrocyanic acid, the easier and more accurate the determination, that organ, if possible, should be selected which has the highest hydrocyanic acid content.

(2) Under field conditions the facilities for conducting a post-mortem examination is often of the poorest, so that the organ should be easily accessible.

(3) The organ should be sufficiently large to enable the execution of more than one analysis in case of accidents happening in the course of the analysis.

With the above in view various organs were analysed. The results are contained in Table 19.

TABLE 19.

The Hydrocyanic Acid Content of various Organs of Sheep Poisoned by Hydrocyanic Acid.

Material.	Mg. HCN/ 100 gm. Sheep 59828.	Mg. HCN/ 100 gm. Sheep 52534.	Mg. HCN/ 100 gm. Sheep 52577.
Ruminal contents	1·84	5·92	1·74
Liver	0·35	0·45	0·19
Lung	0·15	0·15	0·1
Spleen	0·12	0·37	0·21
Kidney	0·09	0·11	0·09
Brain	0·05	0·1	0·05

Specimens of blood were not analysed since at post-mortem examination, it is frequently impossible to obtain blood.

In sheep 52534 and 52577 *tumor splenis* was observed but not in sheep 59828. This is borne out by the relatively much greater weight of the spleen in the first two sheep, namely, 52534 and 52577 as compared with sheep 59828. Waller (1910), and Gettler and Baine (1938) have shown that the blood contains relatively large quantities of hydrocyanic acid so that the relatively much higher hydrocyanic acid content of the spleen of sheep 52534 and 52577 may be ascribed to the increased blood content. The fact that *tumor splenis* does not constantly occur in hydrocyanic acid poisoning renders this organ unsatisfactory for analysis. On the results in Table 19 it was decided to use the ruminal contents and liver for analysis since they showed the highest hydrocyanic acid content and have the added advantage of being easily accessible and even in the case of smaller animals, of sufficient quantity to allow of a number of analyses.

According to Waller (1910) hydrocyanic acid is most abundant in the heart and brain. Gettler and Baine (1938), in poisoning by inhalation, found the lungs to contain approximately twice as much hydrocyanic acid as the brain and kidneys, whilst the two latter organs contained twice as much hydrocyanic acid as the liver. In poisoning *per os* the lungs were again found to contain the most hydrocyanic acid whilst the quantities of hydrocyanic acid found in the liver, brain and kidney approximated each other. There is thus quite an appreciable difference between the results of these authors and the author.

For the establishment of a level of hydrocyanic acid as described prevoiusly it is essential that various portions of the same organ should have the same hydrocyanic acid content. For this reason specimens of various portions of the ruminal contents were analysed and the liver was divided, along lines at right angles to the long axis, into four equal portions, and a specimen of each portion analysed.

TABLE 20.

Hydrocyanic Acid Content of various Specimens of the same Ruminal Contents.

Sheep.	No. 1 Mg. HCN/100 gm.	No. 2 Mg. HCN/100 gm.	No. 3 Mg. HCN/100 gm.	No. 4 Mg. HCN/100 gm.
59828.....	1·84	1·04	2·67	—
52534.....	5·92	4·2	—	—
52577.....	1·74	0·76	1·34	1·1

From the data in Table 20 it is evident that the hydrocyanic acid content of various specimens of ruminal contents of the same animal varies greatly. This is to be expected, because, in the author's experiments, hydrocyanic acid was administered as potassium cyanide in aqueous solution by means of a stomach tube. Consequently the potassium cyanide is introduced into a restricted portion of the rumen. The early paralysis of the rumen and rapid death of the animal preclude an even distribution of the potassium cyanide throughout the rumen. Under field conditions where cyanogenetic plants are ingested it is to be expected that the cyanogenetic plants will either constitute the whole of the grazing, or be more or less evenly distributed