

throughout the grazing. Since death will also probably not occur as rapidly as with a solution of potassium cyanide it is to be expected that various portions of the ruminal contents will contain more equal quantities of hydrocyanic acid.

In cases where poisoning occurs due to the drinking of water contaminated with cyanides a more equal distribution is also to be expected since the large quantity of water consumed will be distributed throughout the rumen. One factor is of importance in this respect, namely, that ruminal contents consist of fluid and solid material. At post-mortem examination, especially if some time has elapsed since death and thus permitting separation of the fluid and solid material, it will be impossible to collect specimens containing fluid and solid material in the correct proportion.

In Table 21 specimen No. 1 represents the extreme left portion of the liver and specimen No. 4 the extreme right portion. It is clear that various portions of the liver vary significantly in their hydrocyanic acid content. It is, therefore, obvious that better results will be obtained if only a certain part of the liver is utilised in all cases. According to Table 21 the right half of the liver shows a more equal distribution of hydrocyanic acid. This statement is statistically correct. It was, therefore, decided to utilise only this part of the liver for all subsequent analyses. The fact that the extreme left portion of the liver may sometimes show a relatively high hydrocyanic acid content may possibly be due to diffusion of hydrocyanic acid through the wall of the reticulum since it is this portion of the liver which is in contact with the reticulum. That fact is very important if specimens are taken some time after death.

TABLE 21.

The Hydrocyanic Acid Content of Various Parts of the same Liver.

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.
50431.....	0.72	0.46	0.46	0.5
60798.....	0.17	0.17	0.2	0.23
60812.....	0.17	0.11	0.14	0.14
52577.....	0.14	0.12	0.18	0.19
60265.....	0.102	0.09	0.09	0.086

Under natural conditions it is unlikely that poisoning will occur in any other way than *per os* so that other methods of introduction of the poison, e.g., by inhalation have not been considered. Where fumigation of houses is carelessly conducted, poisoning of household pets may, however, result by inhalation. The animals were drenched with potassium cyanide in aqueous solution by means of a stomach tube. As soon as death occurred the animals were opened up and the liver and rumen collected in such a way that contamination could not occur. Both the liver and rumen were then immediately transferred to a refrigerator and, after having been chilled for 15 minutes, the necessary specimens were taken and analysed. Animals, which did not receive sufficient potassium cyanide to induce death, were killed by pithing so as to prevent bleeding. Since, as has been mentioned before, the blood contains relatively large quantities of hydrocyanic acid, bleeding would

decrease the blood content and in this way reduce the hydrocyanic acid content of the organs. The organs are chilled prior to analysis in order to minimise the quantity of hydrocyanic acid lost during mincing of the organs.

The result of the analyses are given in Table 22. It is evident that the greater the quantity of hydrocyanic acid administered the higher the hydrocyanic acid content of the liver and ruminal contents. For biological work the correlation between the hydrocyanic acid content of the liver and the quantity of hydrocyanic acid administered is excellent but this is not the case with the ruminal contents. This fact is, however, readily explained by the great variation, already referred to, in the hydrocyanic acid contents of various portions of the ruminal contents of one and the same animal.

TABLE 22.

Hydrocyanic Acid Content of the Liver and Ruminal Contents of Sheep Poisoned by Hydrocyanic Acid.

Sheep.	Weight of Sheep in Kg.	Mg. HCN Administered.	Mg. per Kg.	Mg. HCN/100 Gm. Liver.	Mg. HCN/100 Gm. Ruminal Contents.	Remarks.
50431	31	340	11	0.5	2.4	Collapsed 1½ minutes and died 16 minutes after dosing.
52534	41	460	11	0.45	5.92	Collapsed 5 minutes and died 22 minutes after dosing.
60237	27.8	240	8.6	0.3	1.32	Collapsed 1½ minutes and died 17 minutes after dosing.
60799	29.1	256	8.8	0.24	0.44	Collapsed 1½ minutes and died 18 minutes after dosing.
59828	34.6	230	6.6	0.27	1.84	Collapsed 10 minutes and killed 15 minutes after dosing.
60798	29.1	189	6.5	0.23	1.2	Collapsed 3 minutes and died 21 minutes after dosing.
52577	39.6	170 + 85.0 25 mins. later	6.5	0.18	1.74	Collapsed 25 minutes and died 44 minutes after dosing.
60812	33.2	145	4.4	0.14	0.84	Collapsed 19 minutes and died 43 minutes after dosing.
61480	35.5	128	3.6	0.09	0.76	Severe symptoms and killed 21 minutes after dosing.
60265	28.7	63	2.2	0.09	0.46	Slight symptoms and killed 51 minutes after dosing.

Waller (1910), working mainly on cats; administered hydrocyanic acid by various methods but since organs, other than those analysed by the author, were analysed the results are of no use for comparison. In one instance a liver specimen was analysed in the case of a cat (3.2 Kg.) which had received 10.0 mg. of hydrocyanic acid intravenously. The liver contained 10.05 mg. of hydrocyanic acid per 100 gm.

The quantities of hydrocyanic acid in liver specimens obtained by the author compare favourably with those obtained by Gettler and Baine (1938). The quantities found by these authors in fatal cases of hydrocyanic acid poisoning range from 0.91-0.21 mg. of hydrocyanic acid per 100 gm.

E. THE INFLUENCE OF DECOMPOSITION ON THE HYDROCYANIC ACID
CONTENT OF ORGANS.

Specimens of liver (minced) were placed in distilling flasks ready to be connected for aeration. After being stored for varying periods the flasks were connected to the aeration apparatus in such a way that no hydrocyanic acid could escape. Water, acid, etc., was poured into the flasks in such a way that the gases displaced from the flasks passed through the absorption tubes.

From Table 23 it is evident that, in the case of the liver, putrefaction has a profound influence on the hydrocyanic acid content and in such a way that the majority of the hydrocyanic acid is destroyed in the first twenty-four hours. After twenty-four hours very little further destruction apparently occurs since traces of hydrocyanic acid persist.

TABLE 23.

Effect of Decomposition on the Hydrocyanic Acid Content of Liver.

Mg. HCN/100 gm. Originally Present.	Days of Putrefaction.	Mg. HCN/100 gm. after Putrefaction.
0.29.....	1	0.01
0.22.....	1	0.03
0.22.....	2	0.025
0.45.....	2	0.02
0.35.....	3	0.015
0.35.....	6	0.01

Some of these determinations were done in summer and others in winter so that the degree of decomposition, reached after twenty-four hours, varied in different specimens. Despite the varying degree of decomposition, the results were practically identical in that the hydrocyanic acid had almost completely disappeared after twenty-four hours. In order to study the disappearance of hydrocyanic acid from the liver during the first twenty-four hours, sheep were killed by the administration of potassium cyanide per stomach tube. Immediately after death the liver was removed, minced and thoroughly mixed. Specimens of fifty grams each were taken, one immediately analysed and the rest stored for varying periods.

The results are contained in Table 24. The results are somewhat variable but show that hydrocyanic acid disappears rapidly from the liver after death.

TABLE 24.

The effect of Decomposition on Hydrocyanic Acid Content of Liver.

Sheep.	Mg. HCN/100 gm. Fresh.	Mg. HCN/100 gm. after 5 Hours.	Mg. HCN/100 gm. after 11 Hours.	Mg. HCN/100 gm. after 24 Hours.
61018.....	0.24	0.24	0.1	0.02
61513.....	0.28	0.24	0.19	Less than 0.01.

Although the destruction of hydrocyanic acid is very rapid small quantities of hydrocyanic acid were found to persist in the specimens which were allowed to decompose. This fact may be explained as follows:—

The flasks used in these experiments were of 500 c.c. capacity. The hydrocyanic acid, contained in the liver, is rapidly destroyed by the processes of decomposition. Before destruction is complete, however, some of the hydrocyanic acid escapes from the liver into the air contained in the flask. This hydrocyanic acid, not being in intimate contact with the liver, escapes destruction and persists.

While doing the above determinations, it was observed that on acidifying the contents of the absorption tubes, after converting hydrocyanic acid to thiocyanic acid, relatively large quantities of gas(es), other than sulphuretted hydrogen, were liberated. The possibility existed that these gases may have displaced the hydrocyanic acid from the absorption tubes. Such a possibility was ruled out by the fact that the hydrocyanic acid obtained was always present in the first of the two absorption tubes. In order to make sure a number of absorption tubes were used, ensuring that the last few absorption tubes would be free of the gas(es). This procedure proved that the above results are correct, all the hydrocyanic acid being contained in the first absorption tube.

As pointed out previously various portions of the ruminal contents of the same animal vary greatly in their hydrocyanic acid content. It would, thus, be difficult to determine the effect of decomposition if various specimens of ruminal contents of the same animal are utilised. The following procedure was, therefore, adopted:—50 gm. specimens of ruminal contents, containing no hydrocyanic acid, were placed in distilling flasks and to each added the same volume of a solution of potassium cyanide. One specimen was immediately analysed and the others stored, at room temperatures, in the sealed distilling flasks for various periods. On being analysed the flasks were connected to the apparatus and the necessary water and acid added in such a way that no hydrocyanic acid could escape.

The results are contained in Table 25. From these results it is evident that the majority of the hydrocyanic acid is lost during the first twenty-four hours but that this loss is more gradual than in the case of the liver probably because the processes of decomposition are more active in the liver. As in the case of the liver, small quantities of hydrocyanic acid were found to persist even after the specimens of ruminal contents had been allowed to decompose for several days. The same explanation, as in the case of the liver, may probably be advanced in this case.

TABLE 25.

<i>Days of Putrefaction.</i>	<i>Mg. HCN.</i>
Fresh.....	0.69
1.....	0.17
2.....	0.11
3.....	0.05
5.....	0.05
7.....	0.05

In direct contrast to the above results are those of Gettler and Baine (1938) who found that putrefaction has relatively little effect on the hydrocyanic acid content of liver and brain, only 10.6 per cent. of the hydrocyanic acid being destroyed during 28 days putrefaction of the liver.

The author has very definitely proved that hydrocyanic acid rapidly disappears from the liver after death. The only explanation which the author can suggest is in the light of the work of Mochtar and van Veen (1941), namely, that the original hydrocyanic acid may have been destroyed and replaced by hydrocyanic acid formed by organisms. On the other hand it should be remembered that the maximum quantity of hydrocyanic acid formed during decomposition was found by Gettler and Baine (1938) to be 0.031 mg. per 100 gm.

The results given by Waller (1910) clearly indicate the destructive action of putrefaction on hydrocyanic acid. McNally (1937) states that if the autopsy is not made shortly after death, the presence of hydrocyanic acid may disappear. According to Fröhner (1919) the determination of hydrocyanic acid must be made as soon as possible after death since hydrocyanic acid is readily broken down in the carcass to ammonia and formic acid. The period in which hydrocyanic acid can be found in organs depends, according to Lewin (1929), on the hydrocyanic acid content and the degree of decomposition, although no constant correlation exists between the degree of decomposition and the quantity of hydrocyanic acid found. Buttron (Heffter, 1923), states that the greatest disappearance of hydrocyanic acid in isolated organs occurs during the first twenty-four hours. According to Horn (Heffter, 1923) this disappearance is independent of decomposition; although the latter causes a further disappearance of the poison. Witthaus (1911) states that owing to the great volatility and instability of hydrocyanic acid the probability of its detection in the cadaver diminishes rapidly as time elapses particularly if the conditions favouring putrefaction prevail. According to the same author hydrocyanic acid is hydrolysed to ammonium formate during putrefaction. Witthaus (1911) further states that the statement of Ganassini (Witthaus, 1911) that hydrocyanic acid is practically undetectable in viscera is contradicted by many authors such as Reichardt, Ludwig, Struve and Fagerland who found hydrocyanic acid after relatively long periods after death. Thus Rennard (Lewin, 1929), found hydrocyanic acid after 18 months in a mixture of meat, water and 2.3 gm. of potassium cyanide. Unfortunately the original publications of these authors are not available to the author for it may well be that the relatively long preservation of hydrocyanic acid in these cases may have been due to:—

- (1) the fact that stomach contents were used in which hydrocyanic acid persists for a longer interval;
- (2) that low temperatures may have been involved (European winters);
- (3) that large quantities of hydrocyanic acid may have been present as in the case of Rennard (Lewin, 1929), and that they served as a preserving agent of the organs, and finally;
- (4) that non-specific methods may have been employed so that substances, other than hydrocyanic acid, were determined.

According to Chelle (van Itallie and Bylsma, 1928) hydrocyanic acid is transformed during putrefaction into thiocyanic acid. The work of Magnin (van Itallie and Bylsma, 1928) supports this view but van Itallie and Bylsma (1928) as well as Sensi and Revelloni (van Itallie and Bylsma, 1928) failed to confirm this view. Autenrieth (1928) added bitter almond water or potassium cyanide to organs and blood and found the destruction of hydrocyanic acid to be gradual but points out that hydrocyanic acid has a preser-

vative effect. Crom (Autenrieth, 1928) found that hydrocyanic acid disappears slowly from stomach contents but rapidly from viscera. According to Jollyman (Autenrieth, 1928) the stability of potassium cyanide in the stomach after death is far greater than is supposed.

Steyn (1931) drenched rabbits with the leaves of a cyanogenetic plant *Dimorphotheca spectabilis*. The stomach contents of a rabbit, which had received a sub-lethal dose and was killed 5 hours after drenching, was found to be negative for hydrocyanic acid. A further rabbit was given three minimal lethal doses of the plant and at death the stomach contents were found to be strongly positive for hydrocyanic acid. Of three rabbits, which had received 1½, 2 and 3 minimal lethal doses of the plant and had been allowed to decompose for 20, 30 and 48 hours respectively, the stomach contents were found to be strongly positive. On the other hand the stomach contents of 2 rabbits, which had received 1 minimal lethal dose each of the plant and had been allowed to decompose for 48 hours, were found to be negative for hydrocyanic acid. The above experiments were conducted by Steyn (1931) in December, i.e., during summer so that decomposition was rapid. Steyn (1931) stored the stomach contents of a rabbit, which had received 3 minimal lethal doses of the plant, in a glass-stoppered bottle and found that after 44 days the stomach contents, which had become very acid, were still strongly positive for hydrocyanic acid. In these experiments Steyn (1931) used the sodium picrate test. It is well known that this test is non-specific since any volatile reducing agent will give a positive reaction. The possibility should, therefore, be recognised that, in the experiments of Steyn (1931) and especially in the case where stomach contents were stored for 44 days, substances other than hydrocyanic acid were determined. Steyn (1934a) has improvised an outfit for the use of Government Veterinary Officers for the determination of arsenic and hydrocyanic acid under field conditions. For the determination of hydrocyanic acid the sodium picrate test is employed and in view of the above it is essential to point out that whereas a negative test, in the case of stomach contents, excludes the presence of hydrocyanic acid a positive test does not necessarily prove this poison to be present. A positive test will, therefore, only serve as an indication of the possibility of hydrocyanic acid poisoning so that the necessary specimens should be submitted to a laboratory for analysis.

From the above it is obvious that the consensus of opinion is in support of the results obtained by the author.

In the experiments of the author liver and ruminal contents were allowed to decompose in sealed containers at room temperature. In these experiments the conditions are similar to those in cases in which specimens of liver and ruminal contents are collected immediately after death and placed in air-tight containers and submitted to a laboratory for analysis. Should specimens, however, be collected some time after death has occurred two main factors should be considered (1) decomposition, especially if the carcase is exposed to the sun, will be more rapid than in the above experiments, and (2) diffusion of hydrocyanic acid from the rumen will occur. In connection with the latter a sheep was killed by the administration of potassium cyanide per stomach tube and exposed to the sun for 11 hours.

The animal was then opened and the liver removed. The liver was divided into four equal sections along lines perpendicular to the long axis and a specimen of each section analysed. The results are contained in Table 26.

TABLE 26.

Diffusion of Hydrocyanic Acid into the Liver.

No.	Mg. HCN per 100 Gm.
1.....	0.78
2.....	0.42
3.....	0.3
4.....	0.24

In Table 26 specimen No. 1 represents the extreme left portion of the liver and No. 4 the extreme right. On removal of the liver the peritoneal cavity smelt strongly of hydrocyanic acid. Decomposition of the liver was far advanced so that it would be correct to assume, according to results previously obtained, that at least the major proportion of the hydrocyanic acid, present in the liver at death, had disappeared. Consequently diffusion was responsible for the quantity of hydrocyanic acid present in the liver. Judging from experience the liver at death should have contained approximately 0.25 mg. of hydrocyanic acid per 100 gm., since the animal was given 7.9 mg. of hydrocyanic acid per Kg. From Table 23 it is evident that, as is to be expected, diffusion of hydrocyanic acid from the rumen and reticulum into the liver is greatest in that part of the liver in contact with the reticulum. Furthermore, this diffusion decreases as the distance between the liver and the rumen and reticulum increases.

F. THE EVALUATION OF THE SIGNIFICANCE OF THE HYDROCYANIC ACID CONTENT OF SPECIMENS OF LIVER AND RUMINAL CONTENTS.

It has already been stated that the presence of hydrocyanic acid in the ruminal contents is not definite proof that the animal had died of hydrocyanic acid poisoning because (1) hydrocyanic acid has frequently been found in the ruminal contents of animals which had died from other causes; (2) in the case of cyanogenetic plants the major part of the poison may only have been liberated after death and (3) it does not prove that a lethal quantity of hydrocyanic acid had been absorbed.

The value, therefore, of analysing ruminal contents lies in the fact that it serves as corroborative evidence for the conclusion based on the result of the analysis of a liver specimen. Hydrocyanic acid, being volatile, is readily absorbed so that it can be accepted that, if hydrocyanic acid is present in the stomach contents, part of it has been absorbed. The presence of large quantities of hydrocyanic acid in the ruminal contents will thus indicate that a lethal quantity has been absorbed. Should cyanogenetic plants comprise the source of hydrocyanic acid then, as pointed out before, the possibility should be considered that the hydrocyanic acid may have for the major proportion been liberated after death. Where cyanide solutions constitute the source of hydrocyanic acid this consideration does not apply. The presence of hydrocyanic acid in the ruminal contents will also serve, to a certain extent, to distinguish between poisoning *per os* and by parenteral administration.

According to the data in Table 22 a quantity of hydrocyanic acid in the ruminal contents of 1.0 mg. and over per 100 gm. and a hydrocyanic acid content of 0.14 mg. of hydrocyanic acid and over per 100 gm. of liver should be regarded as indicative of fatal hydrocyanic acid poisoning.

It would, however, not be correct to lay down a hard and fast rule, namely that a hydrocyanic acid content of 0.14 mg. per 100 gm. of liver is definite proof of fatal hydrocyanic acid poisoning since some variation should be expected. According to biological variations one could thus expect to find a hydrocyanic acid content equal and possibly somewhat above this value in non-fatal cases of hydrocyanic acid poisoning and a hydrocyanic acid content somewhat below this value in fatal cases of hydrocyanic acid poisoning. Cases in which the hydrocyanic acid content of the liver approaches the above value should be regarded as border-line cases and in these the anamnesis, symptoms and post-mortem appearances should be carefully considered in order to arrive at a definite diagnosis. Under field conditions the majority of cases will most probably succumb from a number of minimal lethal doses so that in the majority of cases the hydrocyanic acid content will be so far above the above value as to leave no doubt as to the diagnosis.

The author, therefore, bases the diagnosis directly on the hydrocyanic acid content of the liver considering at the same time the hydrocyanic acid content of the ruminal contents as corroborative evidence. Gettler and Baine (1938) adopt a different procedure by calculating the quantity of absorbed hydrocyanic acid from the hydrocyanic acid content of the liver and brain. In this way they determine whether a lethal or sublethal quantity of hydrocyanic acid has been absorbed.

These authors determined the ratio between the quantity of absorbed hydrocyanic acid and the total quantity of hydrocyanic acid in the brain and liver and found the ratio to be approximately 7. The quantity of absorbed hydrocyanic acid was determined by determining the exact quantity of hydrocyanic acid inhaled in cases of poisoning by inhalation and by subtracting the quantity of hydrocyanic acid in the gastro-intestinal tract from the quantity of hydrocyanic acid administered in cases of poisoning *per os*. The quantity of hydrocyanic acid in the brain and liver is calculated from the weight of these organs and the quantity of hydrocyanic acid found on analysis of specimens of these organs. Multiplying this calculated quantity by 7 gives the quantity of absorbed hydrocyanic acid. The absorbed minimum lethal dose of hydrocyanic acid having been determined by Gettler and Baine (1938) it can be stated, whether the calculated quantity constitutes a lethal or sublethal quantity. The following objections can be made to the work of Gettler and Baine (1938):—

- (1) The great variation in the hydrocyanic acid content of the various portions of the ruminal contents makes an accurate determination of the unabsorbed hydrocyanic acid very difficult.
- (2) Of the absorbed hydrocyanic acid, varying quantities, depending on the period of survival, are excreted through the lungs and in the urine.
- (3) The variation in the hydrocyanic acid content of various portions of the liver renders the calculation of the total hydrocyanic acid content difficult.
- (4) It is essential to know the weight of the whole liver and brain. At post-mortem examinations under field conditions it will be impossible to weigh the liver and brain especially when the post-mortem is conducted by laymen. Furthermore it is impracticable to submit the whole liver and brain to a laboratory.

- (5) The ratio of 7 was established on the results of an experiment in which 4 animals were killed with hydrocyanic acid. It would be preferable to extend the experimental work over a greater range from highly lethal to sublethal quantities of the poison to ensure the ratio being valid for all cases of poisoning.

The complicated procedure of Gettler and Baine (1938) would appear to allow opportunities for errors and in the opinion of the author possesses no advantage to advocate its use in preference to the simpler method of the author of basing a diagnosis directly on the hydrocyanic acid content of the liver.

The values of 0.14 mg. per 100 gm. of liver and 1.0 mg. per 100 gm. of ruminal contents apply to those cases in which specimens for analysis are collected immediately after death. Should some time, however, elapse after death before the specimens are collected the following should be borne in mind:—

- (1) Decomposition of organs in the intact animal after death will be more rapid than when the organs are placed in containers at room temperature. In the former case the disappearance of hydrocyanic acid will be accelerated.
- (2) Diffusion of hydrocyanic acid will occur into the liver from the rumen and reticulum so that the hydrocyanic acid content of the liver will not be the same as at the time of death.
- (3) Putrefactive organisms, especially in the liver, may be responsible for the production of hydrocyanic acid.

In such cases the hydrocyanic acid content of the ruminal contents will be affected as follows:—

- (1) Loss of hydrocyanic acid will occur through decomposition and diffusion.
- (2) If cyanogenetic plants are the source of hydrocyanic acid then the hydrocyanic acid content of the ruminal contents may be increased by the continued liberation of hydrocyanic acid from the cyanogenetic glucosides concerned. Such an increase can not occur where cyanide solutions are the source of hydrocyanic acid.
- (3) Certain putrefactive organisms may possibly be responsible for the production of hydrocyanic acid.

The hydrocyanic acid content of the liver will be affected by (1) loss of hydrocyanic acid through decomposition and diffusion; (2) an increase of hydrocyanic acid due to diffusion from the stomach into the liver; and (3) certain putrefactive organisms may be responsible for the production of hydrocyanic acid.

Should decomposition be sufficiently far advanced at the time of collection of specimens one can not but accept that all the hydrocyanic acid present in the liver at time of death has been destroyed so that in such cases it is useless to analyse liver specimens, whatever the hydrocyanic acid content may be (as a result of diffusion), for this amount of hydrocyanic acid can in no way, whatsoever, give an indication of what the original hydrocyanic acid content of the liver had been at the time of death. In such cases

only the hydrocyanic acid content of the ruminal contents can be used in order to arrive at a diagnosis and then only when the above mentioned points have been considered. It is, therefore, obvious that, if specimens are collected some time after death, great difficulty will be experienced in arriving at a diagnosis. It is, therefore, essential, whenever possible, to collect specimens immediately after death.

G. THE PRESERVATION OF ORGANS FOR ANALYSIS FOR HYDROCYANIC ACID.

The fact that decomposition rapidly destroys hydrocyanic acid renders it very unsatisfactory to submit specimens of liver and stomach contents to a laboratory for analysis unless precautions are taken to obviate the effects of putrefaction.

Gettler and Baine (1938) disapprove of the use of formaldehyde in preserving specimens for hydrocyanic acid analysis.

Briese and Couch (1938) introduced mercuric chloride as a preservative for cyanogenetic plants. In view of the success of these authors, the author decided to investigate the possibility of using this preservative for animal organs. Because of the variation in the hydrocyanic acid content of various portions of the ruminal contents the author added similar quantities of hydrocyanic acid to a number of 50 gm. specimens of ruminal contents containing no hydrocyanic acid. To each specimen was added 150 c.c. of water containing 1.0 gm. of mercuric chloride. One specimen was analysed immediately and the others stored for varying periods at room temperature.

The results are given in Table 27. The results prove mercuric chloride to be an efficient preservative agent for ruminal contents containing hydrocyanic acid.

TABLE 27.

Mercuric Chloride as a Preservative for Ruminal Contents.

Material.	Period of Storage.	Mg. HCN Found.
Ruminal contents.....	Fresh.....	0.81
	2 days.....	0.8
	7 days.....	0.82
	11 days.....	0.82
	21 days.....	0.82

Quantities of liver from animals fatally poisoned by hydrocyanic acid were minced and the minced material thoroughly mixed to ensure an equal distribution of hydrocyanic acid. The hydrocyanic acid content was determined as follows:—To specimens of 100 gm. and 50 gm. were added 150 c.c. of water containing 1.0 gm. of mercuric chloride. After suitable periods the specimens were analysed.

Table 28 contains the results. Again mercuric chloride proved to be an effective preservative agent.

TABLE 28.

Preservative Effect of HgCl₂ on Minced Liver.

Mg. HCN per 100 Gram Originally Present.	Period of Storage.	Mg. HCN per 100 gm. after Storage.
0.32.....	6 days.....	0.35
0.45.....	17 days.....	0.41
0.18.....	13 days.....	0.19

Under field conditions it would in many cases be difficult to submit specimens of minced liver. To obviate mincing it was decided to determine what the efficiency of mercuric chloride would be in the case of liver sections. Livers of sheep, poisoned fatally by hydrocyanic acid, were cut into strips 0.5 cm. thick and these strips cut into fairly small sections. The sections were thoroughly mixed to ensure an equal hydrocyanic acid content. This was then determined. To specimens of 100 gm. or 50 gm. were added 150 c.c. of water containing 1.0 gm. of mercuric chloride. These specimens were again stored for various periods before being analysed.

The results are given in Table 29. On section it was observed that the live sections after three days storage showed an outer grey margin about 1.0 mm. wide, whereas the central part still had a slight reddish tinge. In two cases the hydrocyanic acid was determined separately in the mercuric chloride solution and in the liver. It was found that approximately 30 per cent. of the total hydrocyanic acid was contained in the mercuric chloride solution. If some time elapses after death before specimens are collected decomposition will have set in causing a decrease in the hydrocyanic acid content. It was, therefore, necessary to determine whether, in such cases, mercuric chloride would arrest decomposition and prevent any further loss of hydrocyanic acid. To this end the liver was taken from a sheep eleven hours after death, minced, one specimen determined and further specimens (50 gm.) placed in 150 c.c. of water containing 1.0 gm. of mercuric chloride. These specimens were analysed six days later, and on analysis the hydrocyanic acid content of these specimens equalled that of the original specimen.

TABLE 29.

Preservative Effect of HgCl₂ on Whole Liver.

Mg. HCN per 100 Gram Originally Present.	Period of Storage.	Mg. HCN per 100 gm. after Storage.
0.08.....	3 days.....	0.09
0.08.....	6 days.....	0.07
0.29.....	2 days.....	0.3
0.3.....	6 days.....	0.3

Mercuric chloride has, therefore, proved itself to be an efficient preservative of specimens of ruminal contents and liver containing hydrocyanic acid.

H. THE SUBMITTING OF SPECIMENS FOR ANALYSIS.

According to the information contained in the preceding pages the following points should be observed when submitting specimens for analysis:—

(1) Specimens of liver and ruminal contents should be submitted. Of the liver the right half must be used.

(2) The liver should be cut into strips 0.5 cm. wide and these strips cut into small sections. In order to minimise the loss of hydrocyanic acid one strip should be cut at a time and the sections dropped, as they are cut, into the specimen flask containing the mercuric chloride solution. If such a quantity of liver is cut that it is just covered by 150 c.c. of a 1.0 per cent. solution of mercuric chloride it will amount to approximately 100 gm. Whenever possible the weight of the liver should be obtained by subtracting the weight of the specimen flask and its contents before addition of the liver from the weight of the specimen flask and its contents after addition of the liver. If it is possible to obtain the weight of the liver specimen it is preferable that exactly 100 gm. of liver be submitted. It is realized that under field conditions it will be frequently impossible to obtain the weight of the specimen of liver. For this reason 100 gm. quantities of cut liver were added to flasks containing 150 c.c. of a 1.0 per cent. solution of mercuric chloride and weighed after various periods of storage.

Table 30 contains the results. Should unweighed quantities of liver, therefore, be submitted, the approximate original weight of the liver can be calculated from these results. It will be observed that the liver absorbs some of the mercuric chloride solution. On mincing such specimens of liver a large amount of fluid separates from the solid material making it difficult to obtain a specimen containing the correct proportion of solid and fluid material. This difficulty is best overcome by taking a quantity of liver representing 50 gm. and grinding it with sand in a mortar.

TABLE 30.

Effect of Preservation on the Weight of Specimens of Liver and Ruminal Contents.

Days of Storage.	Weight of Liver.	Weight of Ruminal Contents.
0.....	Gm. 100	Gm. 100
1.....	126	97
2.....	128	95
3.....	126	95
4.....	124	96
5.....	123	95
7.....	121	97
8.....	119	95
9.....	117	95
10.....	115	97
11.....	114	—

In connection with the ruminal contents the quantity submitted should be such that it will be well covered by 150 c.c. of the mercuric chloride solution. Again if possible the weight of the specimen must be obtained and

exactly 50 gm. submitted. Since ruminal contents consist of fluid and solid material it will be impossible to obtain the weight of the specimen in a laboratory. The only way to surmount this difficulty is to use only the solid part of the ruminal contents in cases in which the weight of the specimen cannot be obtained immediately. To 150 c.c. of a 1.0 per cent. mercuric chloride solution 100 gm. quantities of solid material from the rumen were added. After being stored for varying periods the material was poured into a funnel and the fluid drained off. The solid material was packed against the side of the funnel and the excess fluid pressed out and allowed to drain off. A quantity of finely divided solid material passed into the fluid and had to be recovered by centrifuging. From Table 30 it is evident that approximately 96 gm. of ruminal contents were recovered. The difference in the weight is, however, so small as to be of no great significance so that it can be considered that the weight of solid material from the rumen undergoes no change on being stored in the mercuric chloride solution. Such specimens are obviously not truly representative but since the specimen of ruminal contents is only analysed to serve as corroborative evidence, satisfactory results will be obtained from such specimens. A further point that should be stressed is that in the case of cyanogenetic plants in the stomach, hydrocyanic acid will continue to be liberated in the mercuric chloride solution since mercuric chloride inhibits, but does not abolish, enzyme action so that the hydrocyanic acid content obtained on analysis will probably not be the quantity of free hydrocyanic acid which existed in the stomach at the time of death.

Since hydrocyanic acid passes into the mercuric chloride solution this must also be analysed. On receipt of the specimen, in the case of liver specimens, the contents of the specimen flask should be poured into a funnel and the mercuric chloride drained off into a measuring cylinder. The weight of the liver is obtained and a quantity weighed off representing 50 gm. of fresh liver. This quantity is then ground in a mortar and to it is added a quantity of the mercuric chloride solution which is in the same proportion to the total volume of the mercuric chloride solution as the quantity of liver taken is to the total quantity of liver submitted. The same procedure is followed in case of the ruminal contents with the exception that a 25 gm. specimen is taken for analysis.

It must, however, be stressed that whenever possible the weight of the specimens should be obtained when submitting specimens, especially where the results of the analyses may be required as evidence in a court of law, for with the above procedure only approximate results will be obtained. Where the weight of the specimens are obtained when collecting the specimens the solid part of the ruminal contents should not be taken only but a more representative specimen. To minimise the loss of hydrocyanic acid the specimen must be added to the mercuric chloride solution before being weighed, thus the weight of the glass container and the 150 c.c. of mercuric chloride solution should be determined before the 100 gm. of the specimens of liver or stomach contents are added.

(3) *Glass containers* only should be used.

(4) Whenever possible the specimens should be collected immediately after death. Where this is impossible the following information should be supplied:—

- (a) the period between death and the collection of specimens;
- (b) the degree of decomposition.

(5) In all cases where specimens are submitted full details of the following should be supplied:—

- (a) symptoms;
- (b) post-mortem appearances;
- (c) the time that elapsed between noticing the animal to be ill and death;
- (d) the source of the hydrocyanic acid.

It is essential to submit specimens of the suspected source of hydrocyanic acid such as plants, water, etc.

VI. CHRONIC HYDROCYANIC ACID POISONING.

As stated previously numerous South African pasture plants are cyanogenetic with the result that pastured animals, at least during spring and summer, continually ingest varying quantities of hydrocyanic acid. This is substantiated by the fact that the presence of hydrocyanic acid was frequently demonstrable in specimens of ruminal contents received at this Institute for routine analysis.

Furthermore in certain areas of the Union of South Africa it is a common practice to feed stock relatively large quantities of the pods of the Camel-thorn tree (*Acacia giraffae*) which has been found to contain relatively large quantities of hydrocyanic acid (Steyn and Rimington, 1935). Other cyanogenetic feeds like linseed cake are also widely used in this country.

Consequently the question arises whether repeated ingestion of small quantities of hydrocyanic acid may cause chronic poisoning and whether such a condition occurs in the Union of South Africa. However, it may be mentioned at this stage that no condition in stock, which provides even a suspicion of the occurrence of chronic hydrocyanic acid poisoning, has, thus far, been encountered in South Africa.

Furthermore, very few references to chronic hydrocyanic acid poisoning could be found in the literature at the author's disposal. Cyanogenetic plants occur in practically every part of the world and the scanty references clearly indicate the improbability of the natural occurrence of such a condition as chronic hydrocyanic acid poisoning in stock.

Niemes (1937) states that the symptoms of chronic Sorghum poisoning differ from those of hydrocyanic acid and advances no reasons for the discrepancy. In the former exaggerated reflexes, inco-ordination of the hind limbs and a slight rise of temperature were observed. In one case inco-ordination of all four limbs was observed. Calves born from affected cows showed complete paralysis and died of starvation or were killed. Since hydrocyanic acid was not proved to be the cause of the symptoms, the above work cannot be considered to furnish conclusive proof of the occurrence of chronic hydrocyanic acid poisoning.

De Gier (1936) describes the following symptoms in horses which had each received 3.0 Kg. of linseed per day for a week: red mucous membranes, a weak but regular pulse, muscular tremors and paresis. Since the horses suffered from laryngitis it was not clear whether the polypnea observed should be grouped with the above symptoms. The linseed contained 18.5 mg. of

hydrocyanic acid per 100 gm. so that each horse received 1.0 mg. of hydrocyanic acid per Kg. of body weight per day. It has been the experience of the author that if subtoxic quantities of hydrocyanic acid are repeatedly administered at short intervals to an animal symptoms of acute hydrocyanic acid poisoning finally develop. It is the opinion of the author that this has been the case in the above since the symptoms described by De Gier (1936) are those of acute hydrocyanic acid poisoning. It is substantiated by the fact that the horses speedily recovered after removal of the linseed from the diet.

Steyn (1932) administered increasing quantities (0.01—0.016 mg. per Kg. body weight) of potassium cyanide to rabbits (daily except Sundays) for a period of twenty-five days. Chronic poisoning did not develop. On the contrary the animals appeared to develop a tolerance.

Lewin (1929) and Kobert (1906) describe various symptoms observed in human beings working with cyanide compounds. Gadamer (1924) simply states that the industrial use of cyanide compounds leads to chronic hydrocyanic acid poisoning with symptoms of head-ache, anorexia and illness. These authors apparently accept, without any definite and conclusive proof, that the symptoms observed were the result of continual exposure to small but toxic quantities of hydrocyanic acid. It is, however, realised that the continual inhalation of hydrocyanic acid is probably more dangerous than the irregular ingestion of cyanogenetic plants. Koelsch and Seligman (Petri, 1930) state that the continued action (mainly industrial) of hydrocyanic acid causes oedematous swelling of the external genitalia and various skin lesions.

Koelsch (Petri, 1930) describes "Acne rosacea", an angioneurotic inflammatory condition of the skin, in chronic hydrocyanic acid poisoning. Since the author was unable to obtain the original publications of the above authors, it could not be ascertained whether the conditions described in man, were indisputably proved to be due to chronic hydrocyanic acid poisoning.

Kobert (1906) describes a case in which twenty-five per cent. of a number of tubercular patients, receiving repeated inhalations of hydrocyanic acid, developed salivation, head-ache, vomiting, slowing of the pulse, lassitude, albuminuria, etc. This affords more direct evidence of the occurrence of chronic hydrocyanic acid poisoning.

Petri (1930), quoting various authors, describes the following changes in hydrocyanic acid poisoning in man:—

- (1) *Acute poisoning.* Initial inflammatory changes in the brain and *medulla oblongata*; acute disease of the ganglion cells; oedema of, and venous stasis and haemorrhages in, the central nervous system with subdural haemorrhages and haemorrhages in the *pia mater*; perivascular haemorrhages and softening especially in the lenticular nucleus.
- (2) *Chronic poisoning.* Degeneration of the peripheral nerves; fatty degeneration and calcification of the smaller vessels of the brain with hyaline degeneration and leucocytic accumulation in the walls of the vessels.

Heffter (1923) states that the repeated administration of hydrocyanic acid causes chronic poisoning but not always with characteristic symptoms, the symptoms frequently appearing to be due to cumulative action.

Collins and Martland (Heffter, 1923) injected rabbits intravenously every other day with 2·0 mg. of potassium cyanide. After 10—16 days the rabbits developed paralysis of the hindlegs with loss of the reflexes and incontinence of the urinary bladder and rectum. The rabbits died 24—60 hours after the appearance of the paralysis. Microscopical examination revealed degeneration of the myelin sheaths of the peripheral nerves; and distinct injury of the cells of the anterior horn of the middle and lower dorsal parts of the spinal cord. In addition these authors describe a case of chronic hydrocyanic acid poisoning in a human being who showed symptoms similar to those observed in rabbits.

Hurst (1940) injected monkeys intramuscularly every day with increasing quantities of a 0·2 per cent. aqueous solution of potassium cyanide. Some of the symptoms were paresis of the legs, cerebellar ataxia, general weakness and recurrent complete blindness. On post-mortem examination the brains of many of the monkeys showed no definite macroscopic changes whilst in other cases readily discernible lesions were observed. On microscopical examination of the central nervous systems of 25 monkeys, 18 showed lesions in the white matter, whilst, except for the presence of fat-containing granular corpuscles and perhaps a few lymphocytes in occasional perivascular spaces, seven revealed no changes. Hurst (1940) summarises the results as follows:—

“ Apart from lesions in the grey matter, which may affect predominantly either the myelin or the nerve-cells or may amount to total necrosis of tissue, areas of partial or complete demyelination, and of actual necrosis may occur in the white matter in the brains of monkeys poisoned with potassium cyanide. Under the conditions of the present experiments, in which large doses of the poison were administered daily, lesions in the white matter were the most obvious and necrosis was perhaps more frequent than mere demyelination. Occasionally single doses of potassium cyanide may produce the same result, but prior intoxication with the drug appears to favour localization of the lesions in the white matter; the clinical findings suggest a summation of the effects of individual doses of the poison. Necrosis may occur suddenly and simultaneously over wide stretches of the cerebral white matter. In distribution the lesions bear considerable resemblance to those in Schilder's disease (diffuse sclerosis), in histological detail to those of acute epizootic leucoencephalitis of horses (McCallum and Buckley). All the signs of acute inflammation may accompany these lesions due to a simple chemical agent.”

Werner (1940) describes a case in which a worker developed acute hydrocyanic acid poisoning. After recovery the following symptoms developed: hypotonia; ataxia; cerebellar disturbances more pronounced on the left than the right side; head-ache; disturbance of the circulation in the hand and feet; and inner deafness. After fourteen weeks these symptoms had mostly disappeared.

Koelsch (van Itallie and Bylsma, 1928) describes the following symptoms in chronic hydrocyanic acid poisoning in factory workers exposed to hydrocyanic acid; head-ache; dizziness; dryness of the throat; sense of pressure in the gastric region; increased haemoglobin value; normal colour index of the blood; and a slightly increased percentage of lymphocytes and basophile leucocytes. He adds that recovery follows rapidly on the discontinuance of the exposure to hydrocyanic acid.

According to Silva (Editorial, 1941) *Holocalyx glaziovii*, a common cyanogenetic weed, is responsible for photosensitisation in cattle. This author suggests that hydrocyanic acid acts by stimulating tissue production of natural porphyrin thus increasing the photodynamic sensitivity of the animal. Unfortunately the original publication is not available to the author so that it is not clear whether hydrocyanic acid was proved to be the etiological agent. The mere fact that the plant contains hydrocyanic acid does not prove this compound to be the etiological agent since the plant may contain a photosensitising agent.

Wieke (1935) describes a case of hydrocyanic acid poisoning in a factory-worker in the nickel-plating industry who was employed with three other workers at a cyanide-bath. Although precautions were taken it was considered that these men were exposed to hydrocyanic acid fumes but no actual determinations of the hydrocyanic acid content of the atmosphere were made. The symptoms observed include a poor appetite; tiredness; oppressive feeling in the chest; vomiting; head-ache; tremors of the arms and legs, and sleeplessness. The blood showed no significant changes. The three other workers developed no symptoms. Wieke (1935) regards the symptoms to be due to changes in the region of the basal ganglia as the result of repeated hydrocyanic acid poisoning. The above case can of course not be considered definitely proved to be due to hydrocyanic acid.

In considering the work of Clark (1936, 1936a, 1937, 1938, 1939, 1940, 1940a, 1940b) the term "pellagra" is used in the same non-committal way as by him.

Clark advances the theory that pellagra is caused by:

- (1) Cyanogenetic substances. This he maintains is the case in Nigeria, Camerouns, Egypt and elsewhere. In these countries cassava, maize and Kaffir-corn (*Sorghum vulgare*) are the foods which are extensively used and are the source of the cyanogenetic substances.
- (2) Other substances e.g. alcohol contained in the diet or substances formed by disordered metabolism which cause an inhibition of cellular respiration similar to hydrocyanic acid. On this basis Clark explains the sporadic cases of pellagra in countries in which the inhabitants exist on diets containing no or very little cyanogenetic substances.

According to Clark the long continued daily ingestion of small quantities of cyanic substances would have the following sequelae amounting to chronic hydrocyanic acid poisoning:—

- (a) Sequelae produced by the direct action of the cyanic substance. These comprise inhibition of the oxidation-reduction processes of the respiratory mechanisms of the blood, glutathione, "Warburg's atmung-ferment", respiratory co-enzyme, cytochromes of the tissue cells, deaminase of the liver, phosphatase of the blood, Vitamins A, B and C, metabolism of the brain, and possibly other metabolic activities.

- (b) Sequelae produced by the metabolic attempts of the body to effect detoxication of the poison. These would follow from the detoxication of hydrocyanic acid by the formation of thiocyanate.

In this connection Clark points out that sulphur is essential for glutathione, tauro-cholic acid, cystine, thyroxine, cerebrosulphuric acid and other substances.

He mentions that the detoxication of hydrocyanic acid leads to a loss of sulphur which cannot be made good, since, besides containing cyanic substances, the diets under consideration are very low in sulphur. The sulphur loss reflects itself in two ways:—(i) There is a decrease of glutathione with consequent inhibition of cellular respiration aggravated by the direct action of cyanic substances. This anoxaemia explains the degenerative changes in the organs, especially the liver and adrenals, seen in pellagrins. (ii) There is a decrease of the cystine content of the dermal structures. This explains the changes observed in the dermal structures of pellagrins.

- (c) Sequelae produced by the substances formed by the processes of detoxication and from the action of products of metabolic disturbances initiated by (a) and (b). The thiocyanate formed will cause a lowering of the blood pressure and in pellagrins according to Clark the blood pressure is lowered.

With the above as basis Clark attempts to elucidate the pathogenesis of pellagra and explains the symptoms and post-mortem appearances of pellagrins as follows:—

- (i) Degenerative changes in organs, especially the liver and adrenals, caused by anoxaemia.
- (ii) Mental changes ranging from cerebation to mania may be due, at least partially, to metabolic disturbances of the brain.
- (iii) Lowered blood pressure may be due to thiocyanate and adrenal damage (cf. low blood pressure in Addison's disease).
- (iv) Dehydration of the tissues may result from adrenal damage since the adrenals control water, sodium and potassium metabolism.
- (v) Dermal pigmentation and irritation may be the result of adrenal damage and sulphur loss (decreased cystine content).
- (vi) Parasitic infestations may, at least partially, be responsible for the gastro-intestinal disorders.
- (vii) Sulphur loss leads to a decreased glutathione content of the blood.
- (viii) Detoxication of the hydrocyanic acid is responsible for the increased thiocyanate excretion.

Thus to Clark chronic hydrocyanic acid poisoning and pellagra are synonymous, but he goes further and would explain coast disease (cobalt deficiency), oat-hay poisoning and damaged sweet clover disease on the basis of cyanic poisoning. Oat-hay poisoning has, however, been shown to be caused by nitrate (Bradley, Eppson and Beath, 1939) whereas the toxic principle from damaged sweet clover has been identified as 3,3'-methylenebis(4-hydroxy coumarin) (Stahmann *et al*, 1941).

Although the arguments of Clark are ingenious and probably in principle correct in some instances, the following fundamental objections to his deductions may be raised:—

- (1) Clark states that “granted the signs observed in these cases (pellagroid diseases of Tropical Africa, etc.) sometimes resemble the effects of a shortage of these and other vitamins but considering the known facts about the protean and disastrous results of even very minute quantities of hydrocyanic acid upon the vital activities of the body, the suggestion that the diet is necessarily deficient in vitamins seems hardly justified in the absence of precise chemical or biochemical proof”.

According to Clark's publications he has not definitely excluded the possibility of a vitamin deficiency before ascribing the disease to some cause other than a deficiency. All he has done is to consider the diet of one region, namely Nigeria and the Cameroons and to state that carbohydrates, fats, mineral salts, Vitamins A, B₁, B₂ (growth factor), C, D and E are sufficient and that in Southern Nigeria protein is no more than minimal in the diet, *whilst in the same area most people must be living near the danger line in regard to the amount of Vitamin B₂ (pellagra preventing factor) in the diet.* It is obvious from his publications that the diets in all the areas under consideration are of the poorest and the fact mentioned by him that pellagra did not occur in England fifty years ago when the diet of the lower classes was very poor is of no significance since different foods were concerned at that time.

- (2) Clark supplies practically no data on the hydrocyanic acid content of the diets concerned.

Cassava is a cyanogenetic plant containing large quantities of hydrocyanic acid (Wehmer, 1931) but since cassava is eaten in the form of “gari”, or when steamed or boiled, the hydrocyanic acid content of the original untreated cassava can give no idea of the actual quantity of hydrocyanic acid ingested. Turnock (1937), using a method which cannot be entirely approved of, found the hydrocyanic acid content of three “gari” samples to range from 0.001 to 0.0015 per cent. Such quantities of hydrocyanic acid appear far too small to cause any serious ill-effects. Depending on what constitutes a minute quantity, one cannot but regard the statement of Clark that even very minute quantities of hydrocyanic acid have “protean and disastrous results” as an exaggeration.

Although Turnock (quoted by Clark) may have demonstrated the presence of hydrocyanic acid in maize, it is not recognised as a cyanogenetic plant of any significance. In support of this contention it could be mentioned that Steyn (1934), who conducted a large number of tests on maize, could only detect hydrocyanic acid in a few instances in the *leaves and stalks* and then only in maize growing on red sandy soil. Specimens of maize (mature seed) analysed by the author contained no hydrocyanic acid.

Sorghum vulgare is a cyanogenetic plant and the seeds (mature and immature) may contain relatively small quantities of hydrocyanic acid as may be seen from Table 15. Again, however, as in the case of cassava the above two foods are prepared in some way or other viz. made into porridge, so that the hydrocyanic acid content of the original material can give no idea of the quantity of hydrocyanic acid actually ingested.

In this connection it will be of interest to refer to the work of Nemoto (1940). He gives the following analyses of manioc (cassava) and manioc flour:—

- Red Manioc 0·0432 per cent. hydrocyanic acid.
- White Manioc 0·0324 per cent. hydrocyanic acid.
- Egg Yolk Sweet Manioc 0·0089 per cent. hydrocyanic acid.
- Sao Paulo Sweet Manioc 0·0116 per cent. hydrocyanic acid.
- Ordinary Manioc Flour 0·0027–0·0037 per cent. hydrocyanic acid.
- Grated Manioc Flour 0·0027–0·0125 per cent. hydrocyanic acid.

Nemoto (1940) further states that all traces of hydrocyanic acid are lost during the bread-making process and that the hydrocyanic acid can be removed from the flour by washing and subsequent pressing or by exposure in thin layers.

Thus in the same way as Clark has failed to rule out the possibility of an avitaminosis B₂ he also failed, due to the absence of analytical data, to prove the diets, referred to by him, to be dangerously cyanogenetic. Furthermore the quantities of hydrocyanic acid should be proved to be sufficient to exert discernible toxic effects.

(3) In his experiments on rats with cyanogenetic diets Clark failed to produce pellagra so that these experiments offer no definite support for his theory, whilst in his experiment on monkeys he produced a pellagra-like condition which he ascribed to hydrocyanic acid derived from *dhurra*. The control monkey which received the same diet, with the addition of dates, did not develop the condition and this Clark ascribes to the high sulphur content of the dates. The following objections, however, can be made to these conclusions:—

- (i) The same remarks as have been made previously in connection with *Sorghum vulgare* also apply here.
- (ii) The fact that dates contain a large amount of sulphur does not necessarily mean that the prevention of the condition by dates can be ascribed to that substance.
- (iii) No data on the sulphur content of the diet, in the absence of dates, are given for it may well be that the diet contains sufficient sulphur for the needs of the body and for the detoxication of the quantities of hydrocyanic acid concerned, so that the sulphur in the dates may simply be in excess.
- (iv) It is a well known fact that care should be exercised in forming an opinion on the result of an experiment in which only one experimental animal is employed.

In view of the above objections this work of Clark should be regarded with reservation and does not bring us very much nearer the solution to the problem of chronic hydrocyanic acid poisoning, although it must be admitted that his arguments in some instances are probably sound.

According to Kennedy and Purves (1941) seeds of the following have been found to contain a goitrogenic agent:—Swede, soft turnip, hard turnip, rape and chou moellier.

According to Webster *et al* (1931) spring and summer cabbage is goitrogenic to a lesser degree than autumn and winter cabbage and further that the goitrogenic activity is greater in some years than others. They state that this variation is in part dependent on variation in the amounts of goitrogenic and anti-goitrogenic agents in the plant.

Marine *et al* (1933a) state that the press juice of steamed cabbage has no goitrogenic effect, whereas cabbage cake has a marked effect which can be enhanced by washing the cabbage cake with distilled water. These authors (Marine *et al*, 1929) are of the opinion that the goitrogenic agent acts by inhibiting some oxidation system which the thyroid attempts to overcome, resulting in the exhaustion of the thyroxine store of this gland and in this way induces a relative iodine deficiency and hyperplasia of the thyroid.

Since isothiocyanates (mustard oils) are the most characteristic constituents of *Brassicaceae*, Marine *et al* (1932) thought that these compounds or their cyanide precursors may constitute the goitrogenic agents. Allyl-, ethyl-, and phenyl-isothiocyanates were, however, fed to rabbits with negative results. Since Hoffman (Marine *et al*, 1932) isolated nitriles from several of the *Cruciferae* these authors considered it possible that cyanides may be the goitrogenic agents. Marine *et al* (1932), therefore, tested allyl-, propyl-, phenylaceto-, phenylpropyl-, and benzonitrile as well as phenylisocyanide, potassium cyanide, cyanamide and sodium thiocyanate. They found that goitre is produced in rabbits by all the cyanides in varying degree, acetonitrile being most active and the aromatic nitriles, as a group, the least active. Cyanamide was found to produce only a slight reaction and sodium thiocyanate no reaction at all. It is not clear whether the statement "by all the cyanides" includes potassium cyanide or only the nitriles. Marine *et al* (1932) state that the difference in the reactions produced by the cyanides is not altogether due to the differences in the doses of cyanide. The authors add that the goitrogenic property of the above compounds could not be counteracted by administering sodium thiosulphate intra-peritoneally. They, furthermore, state that their experiments have demonstrated that substances which depress oxygen consumption may increase thyroid activity and that cyanides are among the most potent of these goitrogenic agents. A cyanide has not been definitely isolated from cabbage but according to Marine *et al* (1932) enough evidence is available to support the contention that the goitrogenic activity of the plant is due to a cyanide. Spence *et al* (1933) state that the production of thyroid hyperplasia by means of organic cyanides is dependent entirely on the gradual liberation of hydrocyanic acid in the organism so that organic cyanides which are relatively stable (such as phenylcyanide) will produce no thyroid reaction. They also point out that a negative result will be obtained in animals, such as chickens, in which hydrocyanic acid is liberated only to a very small degree even from such a nitrile as methyl cyanide. According to Hunt (Spence, 1933) the cyanide action of organic cyanides depends on the liberation of the cyanide-group; the more rapidly the cyanide group is liberated, the more toxic is the organic cyanide. According to Adeline *et al* (1926) the toxicity of aliphatic cyanides is directly proportionate to the rate of liberation of hydrocyanic acid. They, however, state that the toxicity of benzyl and phenyl cyanides is not proportional to the rate at which they split off hydrocyanic acid but seems to be a highly specific property of these compounds.

James (1939) points out that the goitrogenic properties of cabbage is not surprising if it is considered that Hoffman (James, 1939) demonstrated