the presence of nitriles in members of the sub-family *Brassica* for a well-known method of biological standardisation of thyroid preparations is by feeding mice with aceto-nitrile and the thyroid preparation whose value is to be assessed. The properties ascribed to the goitrogenic agent in cabbage by Marine *et al* (1929), Marine *et al* (1930), Webster and Cipra (1930), Bauman *et al* (1931) and Webster *et al* (1931) are according to James (1939) readily explained by the known chemical behaviour of nitriles. James (1938) found 1·6–8·6 mg. of cyanide, calculated as sodium cyanide in the urine of persons who at the meal immediately prior to the experiment had partaken of cabbage and Brussels sprouts. Prior to the meal the urines were free from cyanide.

Marine *et al* (1933) found that the feeding of fresh vegetables markedly inhibits the goitrogenic activity of methyl cyanide. Spence *et al* (1933) produced goitre in rabbits by means of cabbage and methyl cyanide and point out that it is important to consider the diet of the animals, for anti-goitrous substances may be present and, if present in sufficient quantities, may prevent the development of goitre. Marine *et al* (1933a) state that in solution in the press juice of cabbage is a substance inhibiting the goitre-producing agent and which is removed or destroyed by washing of the cabbage-cake. Further work by these authors showed that this anti-goitrous substance was not iodine, that it could be roughly measured by its capacity to absorb iodine and that it may be identical with hexuronic acid.

McCarrison (1933) found soya beans to be goitrogenic. Sharpless *et al* (1939) found a diet containing soya bean flour to be goitrogenic. They state that soya beans are relatively rich in cyanogen and yield considerable quantities of cyanide on enzyme hydrolysis and that this may possibly account for the goitrogenic activity of soya bean flour. Ranganathan (1933) obtained by hydrolysis with 3 per cent. sulphuric acid 6·3 mg. and 4·1 mg. of hydrocyanic acid (calculated as cyanide) per Kg. in cabbage and soya beans respectively.

Extensive work has, thus, already been done on goitre in relation to hydrocyanic acid but it is obvious that much work remains to be done. From the literature quoted it is evident that:—

(i) Cabbage and other members of the sub-family *Brassica*, as well as soya beans, possess goitrogenic properties. Much evidence has been accumulated that the goitrogenic agent concerned is an organic cyanide (nitrile) but conclusive proof is still lacking.

(ii) Organic cyanides are capable of inducing thyroid hyperplasia. So far only Marine *et al* (1932) have considered inorganic cyanides, namely potassium cyanide but, to the author, it is not clear from their publication, whether this compound yielded positive or negative results.

(iii) Goitre as a result of the ingestion of these cyanide compounds and foodstuffs have been so far only produced experimentally and no reference in the literature available to the author could be found to the natural occurrence of goitre as a result of ingesting them.

This may be due to:—

(i) Natural diets may contain an insufficient quantity of the goitrogenic foods. In the experimental diets these foods constituted the major or a large proportion of the ration.

(ii) Normal diets, if they do contain goitrogenic foods in sufficient quantities, may also contain sufficient anti-goitrogenic agent to neutralise the action of the former.

Onderstepoort Experiments.

Because of the unsatisfactory state of our knowledge of chronic hydrocyanic acid poisoning the author conducted the following experiment:—

A number of ewes, some of which served as controls, were run in a small camp with a stable adjoining to provide shelter against extremes of weather. The size of the camp was such that it permitted of sufficient exercise.

The sheep were placed on a ration of lucerne hay and crushed maize with veld hay *ad lib.* Whenever available, green feed (lucerne, barley, oats) was given and occasionally maize silage was supplied.

Before the commencement of the experiment the sheep were treated for internal parasites and thereafter treatment for worms was applied at regular intervals. In order to ensure that the sheep were actually free from internal parasites Dr. Mönnig of the Institute kindly had faecal cultures made of all the sheep at regular intervals.

The sheep were dosed per stomach tube twice daily at 9 a.m. and 3.30 p.m. with an aqueous solution of potassium cyanide except on Sundays and public holidays. On Saturdays only one dose was given at 9 a.m. The hydrocyanic acid solution used at first contained a quantity of potassium cyanide equivalent to 1·1 mg. of hydrocyanic acid per c.c. and subsequently a solution containing a quantity of potassium cyanide equivalent to 1·6 mg. of hydrocyanic acid per c.c. At first the sheep received a quantity of potassium cyanide corresponding to 1·1 mg. of hydrocyanic acid per Kg. of bodyweight which corresponds to one-half of the minimal lethal dose given by Steyn (1934) as 2·2 mg. of hydrocyanic acid per Kg. of bodyweight. The above quantity, however, appeared to be very near the minimal toxic dose so that the quantity of potassium cyanide given was increased to correspond with 1·6 mg. of hydrocyanic acid per Kg. of bodyweight which appeared to be the maximum toxic but non-lethal dose. At the same time an attempt was made to dose the sheep three times a day at 9 a.m., 12 noon and 3.30 p.m., but after the first day it was evident that this would result in acute fatal hydrocyanic acid poisoning and it was therefore decided to administer the hydrocyanic acid twice daily.

With a dose of 1·1 mg. of hydrocyanic acid per Kg. of bodyweight in the form of potassium cyanide severe symptoms were rarely seen. Slight acceleration of the pulse and respiration with sometimes a slight degree of apathy were the usual symptoms. With a dose of 1·6 mg. of hydrocyanic acid per Kg. of bodyweight the symptoms observed were often severe and consisted of apathy, severe dyspnoea, accelerated pulse and twitching of the lips. Not infrequently a sheep would show ataxia and finally collapse in which case the sheep was given 4·0 gm. of sodium thiosulphate intravenously in a 20 per cent. aqueous solution to prevent death occurring. Without treatment the symptoms usually lasted about 30 minutes and in the extreme cases recovery after the thiosulphate injection was extremely rapid. If the same sheep persisted in showing extremely severe symptoms the dosage was slightly decreased. The animal was then gradually brought on to the full dose again in the course of a few days.

A peculiar symptom, which was often observed in extreme cases, was a drawing back of the head and often a little sideways, the sheep moving backwards.

The above symptoms were usually observed a few minutes after dosing but were often long delayed. In this respect the pH of the ruminal contents appeared to be a determining factor, namely if acid, hydrocyanic acid is liberated from the potassium cyanide and rapidly absorbed.

The ruminal contents may, however, be neutral or alkaline and only subsequently become acid. Potassium cyanide, being non-volatile, is absorbed slowly, if at all, in the rumen.

The sheep were weighed every month and the quantities of potassium cyanide administered, adjusted according to the fresh weights.

Occasionally a sheep died and was then replaced by another. At monthly intervals during the first year of the experiment, and subsequently when new sheep were introduced into the experiment, red cell counts and haemoglobin determinations were made of some of the sheep receiving potassium cyanide and of some of the control sheep. For the red cell counts a Bürker-Turk haemocytometer was employed. The haemoglobin determinations were first done by the Newcomer method but were subsequently kindly done by Mr. G. C. S. Roets of this Institute using the iron method (Roets, 1940).

At the beginning of each year rams were run with the ewes for a period of six weeks in order to ascertain whether hydrocyanic acid had any detrimental effect on the fertility of the animals, pregnancy, or on their progeny. The lambs were not given any hydrocyanic acid. In addition to sucking their mothers they had free access to the food of their mothers. The ewes were allowed to wean their lambs themselves.

The case reports of all the experimental and control sheep are given in detail in Appendix I. According to them the following observations may be made:—

(1) The quantities of hydrocyanic acid given to the experimental sheep are recorded in Table 31.

TABLE 31.

Quantities of Hydrocyanic Acid given to the Experimental Sheep.

Sheep.	Period during which 1·1 Mg. of HCN per Kg. of Bodyweight was given twice daily.	Period during which 1·6 Mg. of HCN per Kg. of Bodyweight was given twice daily.
52361	4 months	—
53203	2 months	17 months.
53163	15½ months	3¼ months.
53051	15½ months	10 months.
52511	15½ months	15 months.
52445	15½ months	20 months.
59827	—	6½ months.
59828	—	6½ months.
59473	—	6½ months.
59871	—	6½ months.

(2) Of the experimental ewes five died. Of these, four died suddenly without any previous signs of illness. In this connection it must be pointed out that (*a*) the animals were receiving maximal toxic doses of potassium cyanide with the exception of ewe 52361; (*b*) they were watched after dosing for a short period but that sometimes the appearance of symptoms after dosing was delayed. It is, therefore, fairly definite that the above animals died of acute hydrocyanic acid poisoning. That some of the experimental ewes should have died of acute hydrocyanic acid poisoning is not at all surprising since extremely severe symptoms of hydrocyanic acid poisoning were repeatedly observed in the experimental animals. In many cases the symptoms were so severe that treatment with sodium thiosulphate had to be resorted to in order to prevent the animals from dying. (See Appendix II.) On a few occasions severe symptoms were observed when the dosage was 1·1 mg. of hydrocyanic acid per Kg. of bodyweight and consequently ewe 52361 may also have died of acute hydrocyanic acid poisoning.

In the case of ewe 53163 macro- and microscopical examination did not reveal the cause of the paralysis which the ewe gradually developed. Considering the work of Collins and Martland (Heffter, 1923) and Hurst (1940) it appears probable that it may represent a case of chronic hydrocyanic acid poisoning. Since, however, the animal was the only one of the ten experimental animals, which became paralysed, it is not justified to draw any definite conslusions.

(3) Mr. H. P. A. de Boom, of this Institute, who kindly undertook the histological examination of the specimens from the experimental ewes that died, summarises the histological changes observed as follows:—

Liver.—Slight to marked vacuolar degeneration with which may be associated fatty degeneration or infiltration.

Adrenal.—A decrease in, or, an almost complete disappearance of, sudanophile fats from the cortex was observed, but in one case an increase of these fats was seen in the outer cortical zone.

Kidney.—Slight to marked fatty changes accompanied by slight haemosiderosis and bile pigmentation, in one case, are the only significant changes.

Spleen.—No changes attributable to hydrocyanic acid poisoning were seen.

Myocardium.—Fatty or parenchymatous changes constitute the only significant changes observed.

Hypophysis.—In one case there is a marked colloid degeneration of the *pars intermedia.*

Nervous system.—The only lesion of any possible significance is a minor haemorrhage observed in one case in the cerebral cortex where the surrounding tissue had undergone necrobiotic changes.

From the above it is evident that the histological changes are not constant in all the animals and in no way correspond to the changes described by Petri (1930), Collins and Martland (Heffter, 1923) and Hurst (1940). Clark (1939) described the following changes in humans on diets containing cassava or maize:—

Liver.—Fatty and destructve degeneration accompanied by haemorrhages, pigmentation and bile stasis.

Adrenals.—Especially the *zona fasciculata* is affected. The changes comprise vascular congestion, fatty degeneration, haemorrhages, pigmentation and atrophy.

Kidneys.—Congestion, cloudy swelling, pigmentation and the presence of calcium crystals in the collecting tubules constitute the changes observed. The histological picture described by Clark (1939) shows, therefore, very little similarity with that described above.

(4) In comparison with the controls, the weights, red cell counts and haemoglobin values of the experimental animals did not show any significant changes. (See Appendix III.)

(5) At post-mortem examination the thyroids of the experimental animals that died or were killed showed no significant changes. The thyroids of the other experimental animals revealed nothing unusual on clinical examination.

(6) Of the first lamb crop 2 lambs were born from experimental ewes and 3 from control ewes. At this time there were 4 experimental and 5 control ewes. One of the control ewes suffered from dystokia and her lamb was born weak and it died 3 days later.

Of the second lamb crop 5 lambs were born from control ewes and 3 from experimental ewes. At this time there were 5 control and 3 experimental ewes. Two of the lambs born from control ewes died. One died after its mother had succumbed from mastitis. The other lamb was born weak (difficult parturition) and remained so till it died one month later.

Of the third lamb crop two lambs were born from experimental ewes and two lambs from control ewes. At this time there were six experimental and three control ewes.

All the lambs born from experimental ewes were normal at birth and their subsequent progress did not show any significant variation from that of the lambs born from control ewes. From the above it is also evident that the fertility of the experimental ewes approximately equalled that of the control ewes.

The work of the author proves that chronic poisoning by hydrocyanic acid does not occur in a form similar to chronic poisoning by minerals such as arsenic or lead in which even small fractions of the lethal dose induce chronic poisoning when they are administered over long periods.

This fact can probably be explained by the rapid elimination of hydrocyanic acid and its rapid transformation into compounds such as thiocyanate. The work of such authors as Niemes (1937), Koelsch (Petri, 1930), Koelsch and Seligman (Petri, 1930), Lewin (1929), Gadamer (1934) and Wieke (1935) can only be considered as possible circumstantial evidence of the occurrence of chronic hydrocyanic acid poisoning since, in the cases concerned, hydrocyanic acid was accepted to be the etiological agent and not definitely proved to be such. From the literature it is, however, evident that experimentally chronic hydrocyanic acid poisoning has been produced in the form of two widely different symptom-complexes.

A. The work of Collins and Martland (Heffter, 1923) and Hurst (1940) proves that chronic hydrocyanic acid poisoning may be produced experimentally. In this connection it must be remembered that:—

(1) Potassium cyanide was not administered *per os* but intravenously and intramuscularly, and that

(2) relatively very large quantities were administered inducing a severe degree of poisoning.

It would, therefore, appear that, especially in cases of parenteral administration, severe poisoning by hydrocyanic acid may inflict damage on the central nervous system and that the sum total of the damage sustained from repeated poisoning of such a severe degree may be responsible for a condition which could be regarded as chronic hydrocyanic acid poisoning. Such a condition, however, is obviously extremely unlikely to occur naturally since no farmer would voluntarily allow his stock to become, in a short period, repeatedly severely poisoned by hydrocyanic acid. In this connection it should again be stressed that, although cyanogenetic plants are very prevalent in the Union of South Africa, no evidence has been encountered even to suspect the occurrence of chronic hydrocyanic acid poisoning in stock grazing on such pastures.

B. Marine *et al* (1932) have demonstrated the goitrogenic property of hydrocyanic acid in various combinations, especially in the form of organic cyanides. The goitrogenic agent in cabbage and other members of the subfamily Brassica is considered to be an organic cyanide. Two reasons have already been given by the author why under natural conditions goitre is not likely to be encountered as the result of the action of these compounds, namely:—

(1) In the experimental production of goitre the goitrogenic food constitutes the major or large proportion of the diet which is unlikely to occur naturally, and

(2) the anti-goitrogenic agents contained in many feeds will serve to prevent the development of goitre.

The author, therefore, appears justified to conclude that, although chronic hydrocyanic acid poisoning may be produced experimentally, it is not likely to occur under natural conditions, except in isolated cases.

VII. SUMMARY.

Of the various methods the ferric thiocyanate method is selected for the determination of hydrocyanic acid both in plants and in animal organs. It is shown that erroneous results may be obtained in certain plants by the alkaline titration method. The ferric thiocyanate method is dealt with in detail and several modifications introduced. It is demonstrated that the method yields excellent results. For the liberation of hydrocyanic acid from the material to be analysed the aeration procedure is adopted as it yields the best results.

The sources of hydrocyanic acid in cases of poisoning in stock are discussed and the results of the examination of a large number of plants for hydrocyanic acid are given.

The factors concerned in the causation of poisoning of animals by cyanogenetic plants are discussed in detail and these factors include the quantity of the plant ingested; the previous diet of the animal; the hydrogen-ion concentration of the stomach contents; the percentage of the total hydrocyanic acid in the plant which is present in the free state; the quantity of enzyme present in the plant; and the hydrocyanic acid content of the plant. In connection with the last-named factor, a detailed discussion is given on the manner in which it is influenced by the following: soil; climatic conditions; age of the plant; variety of the specimen; individual variation; part of the plant; and the state of the plant. The variability of the hydrocyanic acid content of cyanogenetic plants is illustrated by graphical representations of the hydrocyanic acid content, over a number of days, of *Dimorphotheca Ecklonis* and four strains of *Cynodon plectostachyum*. The effect of wilting on the hydrocyanic acid content is clearly shown. It is pointed out that the danger, which a cyanogenetic plant constitutes to stock, should be judged on the maximum quantity of hydrocyanic acid which can be developed by the plant.

The physiological significance of cyanogenesis in plants is discussed.

The ruminal contents and livers of sheep which had died at this Institute from causes other than poisoning by hydrocyanic acid were found to contain only traces of hydrocyanic acid. Hydrocyanic acid was, however, frequently demonstrable in relatively large quantities in the ruminal contents of animals, which had died from causes, other than hydrocyanic acid poisoning, under field conditions. Of the organs of sheep which were poisoned by hydrocyanic acid *per os*, the ruminal contents and liver contain the largest quantities of hydrocyanic acid of the organs analysed. The quantity of hydrocyanic acid ingested is shown to be reflected in the hydrocyanic acid content of the ruminal contents and liver. The hydrocyanic acid content of the liver and ruminal contents of approximately twenty sheep which had received varying quantities of hydrocyanic acid was determined. In the case of the liver the hydrocyanic acid content was 0·14 mg., or more, per 100 gm. in sheep which had received lethal quantities, or less than 0·14 mg. per 100 gm. in sheep which had received sublethal quantities of hydrocyanic acid *per os*. The corresponding value for the ruminal contents is approximately 1·0 mg. of hydrocyanic acid per 100 gm. The relative value of the hydrocyanic acid contents of the liver and ruminal contents in arriving at a diagnosis of hydrocyanic acid poisoning is discussed. It is shown that the hydrocyanic acid content of the ruminal contents serves as corroborative evidence for the hydrocyanic acid content of the liver and that it is essential to know the latter in order to arrive at a definite diagnosis. During decomposition of organs of animals, not poisoned by hydrocyanic acid, significant quantities of hydrocyanic acid did not develop. It is demonstrated that hydrocyanic acid rapidly disappears from the liver but more gradually from the ruminal contents when they are allowed to decompose. Mercuric chloride was found to be an excellent preservative of ruminal contents and liver and completely prevented the disappearance of hydrocyanic acid from these materials during storage. This fact renders it possible to submit specimens of liver and ruminal contents to a laboratory for analysis. It was demonstrated that, in the intact animal, considerable diffusion of hydrocyanic acid occurs from the stomach contents into the liver after death.

The problem of chronic hydrocyanic acid poisoning is fully discussed. The experimental work of the author did not result in the production of

chronic hydrocyanic acid poisoning in sheep. From the literature on the subject, available to the author, it is evident

(1) that some authors have produced chronic hydrocyanic acid poisoning in the form of injury to the central nervous system in rabbits and monkeys, and,

(2) that other authors have demonstrated the goitrogenic activity of hydrocyanic acid in various conbinations.

It is shown, however, that the experimental conditions for the production of these forms of poisoning are such that it is very unlikely that they will occur naturally in stock.

VIII. CONCLUSIONS.

(1) The aeration procedure is the most suitable for the liberation of hydrocyanic acid from biological material.

(2) The ferric thiocyanate method, as modified by the author, is an accurate and sensitive method for the determination of hydrocyanic acid in biological material.

(3) In the case of certain plants erroneous results were obtained by the alkaline titration method.

(4) Hydrocyanic acid was demonstrated in a number of plants.

(5) The hydrocyanic acid content of cyanogenetic plants varies greatly, one of the most important factors, responsible for the variation, being climatic conditions. Wilting is responsible for a great increase in the hydrocyanic acid content of cyanogenetic plants.

(6) The danger of a cyanogenetic plant to stock should be assessed on the maximum quantity of hydrocyanic acid which the plant may develop.

(7) Under field conditions hydrocyanic acid, in relatively large quantities, was frequently demonstrable in the ruminal contents of animals which had died from causes other than hydrocyanic acid poisoning. Under laboratory conditions ruminal contents and liver of such animals, on a practically non-cyanogenetic diet, contained only traces of hydrocyanic acid.

(8) In sheep poisoned by hydrocyanic acid *per os* the ruminal contents and liver show the highest concentration of hydrocyanic acid of the organs analysed.

(9) The quantity of hydrocyanic acid ingested is reflected in the hydrocyanic acid content of the ruminal contents and liver.

(10) Since the presence of hydrocyanic acid in the ruminal contents does not prove that a lethal quantity of hydrocyanic acid was absorbed a definite diagnosis cannot be made on the hydrocyanic acid content of the ruminal contents alone. The hydrocyanic acid content of the ruminal contents can, therefore, only serve as corroborative evidence for the hydrocyanic acid content of the liver.

(11) The livers of sheep given lethal quantities of hydrocyanic acid contained 0·14 mg., or more, of hydrocyanic acid per 100 gm., whereas the ruminal contents contained approximately 1·0 mg., or more, of hydrocyanic acid per 100 gm.

(12) During storage a decrease occurs in the hydrocyanic acid content of liver and ruminal contents, the decrease being very rapid in the former and more gradual in the case of the latter.

(13) Mercuric chloride is an excellent preservative of ruminal contents and liver, entirely preventing the decrease in the hydrocyanic acid content during storage.

(14) Under the conditions of the author's experiments hydrocyanic acid did not develop in significant quantities during decomposition of liver and ruminal contents.

(15) In the intact animal after death hydrocyanic acid diffuses to a considerable degree into the liver from the ruminal contents.

(16) Chronic poisoning by hydrocyanic acid does not occur in a way similar to chronic poisoning by mineral poisons such as lead.

(17) Experimentally, hydrocyanic acid has been found to cause :—

(*a*) Chronic poisoning in the form of injury to the central nervous system and

(*b*) goitre.

The conditions under which these forms of poisoning were produced are such, however, that it is very unlikely that these forms of poisoning will occur naturally.

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XI. APPENDICES.

APPENDIX I.

The following are the case reports of all the experimental and control sheep : —

Sheep 53163 was given 1·1 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 15¼ months followed by 1·6 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 3¼ months.

Symptoms.

The ewe developed weakness of the hindquarters which gradually increased till she was completely paralysed a week later. During the last few days anorexia and emaciation were observed. The ewe was killed *in extremis.*

Post-mortem Appearances.

Emaciation; patchy atalectasis of the right lung; regressive changes in the liver; inflammation of the frontal sinus; slight congestion of the blood vessels of the meninges.

Histology.

Liver.—There is vacuolation of all the hepatic cells. Small fat droplets are distributed in small numbers throughout the liver but are more numerous at the periphery of the lobules. In addition there are numerous large fat drops, many of which show an eccentric vacuolar space. One small focus of neutrophile accumulation was seen.

Myocardium.—Fine fatty globules are diffusely spread throughout the myocardial musculature.

Spleen.—Normal.

Kidney.—Fairly large fat droplets are present in the epithelium mainly of the ascending limb of Henle's loop and of the distal convoluted tubules.

Adrenal.—There is an increased lipoid content of the cells in the outer zone of the cortex ("*glomerulosa*") as evinced by the presence of numerous large globules of sudanophile material. Occasional fatty globules are noted in the endothelial cells. The cytoplasm of these cells often contain large vacuoles. Especially in the epithelial cells of the *fasciculata*, rounded or oval cytoplasmic bodies were noted lying in vacuoles, in such a manner that each body is surrounded by a clear halo-like space. These bodies stain somewhat similarly to the cell cytoplasm but are more homogeneous and hyaline in appearance and more eosinophilic in their reaction to haemalum-eosin. Superficially there is a striking resemblance to inclusion bodies. Such structures have been described in the epithelial cells of the kidneys of animals (rabbits) poisoned by bismuth.

Nervous System.—Neither Sudan III nor Marchi methods succeeded in revealing any definite lesions in the cerebrum, midbrain, cerebellum, medulla oblongata, various levels of the spinal cord and in the sciatic nerves.

Sheep 52445 was given 1·1 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 15½ months followed by 1·6 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 20 months. The animal was killed for the collection of specimens. Throughout the course of the experiment nothing abnormal was observed in the animal.

Post-mortem Appearances.

Pyometra, submucosal haemorrhages on the turbinate bones.

Histology.

Gastro-intestinal Tract.—Normal.

Liver.—The liver is normal except for a small number of fat globules contained in the hepatic cells in scattered foci usually peripheral in situation. The globules are mostly fairly large, indicating a fatty infiltration. They may contain a large eccentrically placed vacuole which is refractive to staining. A few minute globules are also present as are granules of bile pigment in uniform distribution.

Pancreas.—Normal.

Lung.—No abnormality was noted except for a diffuse fibrosis of the alveolar walls in as much as collagenous fibrils have been deposited around the capillaries in irregular bundles. This condition has been observed fairly frequently in sheep at this Institute.

Myocardium.—No abnormality was noted except for the presence of sarcosporidial cysts in small numbers.

Spleen.—Nothing abnormal was seen.

Lymph Gland.—There is a fairly marked reticulo-endothelial mobilization with clumps of histiocytes forming fat-laden foci in the cortex near the medulla. Plasma cells are numerous as well as eosinophiles. There is a relative paucity of secondary follicles. Another section presents a similar picture except for the lesser number of plasma cells and eosinophiles.

Kidney.—Autolysis has already advanced. Nothing unusual was noted except for a few calcareous deposits in the interstitium and walls of the venules near the apex of the renal pyramid. This renal chalicosis is of common occurrence in sheep.

Epiphysis.—Normal.

Hypophysis.—Large cysts crammed with colloid are present in the *pars intermedia*. Some have enlarged to such extent that the surrounding tissues have undergone pressure atrophy and even necrobiosis with haemorrhage so that some colloid has become liberated into the cleft.

Adrenals.—There is an almost complete disappearance of sudanophile fats, a few globules and larger drops still being present in the cells of the "*glomerulosa*" and still less in that part of the *fasciculata* bordering on the reticulate zone. In the outer zone and to a lesser extent in the *fasciculata* large vacuoles occur in the cells. Some of the fat droplets also exhibit eccentric vacuolation. These latter "degenerative" changes, described in many types of septicaemia in the human, can also be observed in histological preparations of the adrenals of normal sheep

Central Nervous System.—A number of small haemorrhages of recent origin were found, namely, one in the outer (molecular) layer of the cerebral cortex. The tissues in this case show evidence of necrobiosis and commencing liquefaction and one neuron is affected as shown by the commencing neurophagocytosis. A further haemorrhage occurred in the thalamus, and in this case a few neutrophiles have migrated into the haemorrhage. A number of haemorrhages are present in the *formatio reticularis* of the medulla oblongata and a few in the dorsal horn of the grey matter of the thoracic spinal cord. Further haemorrhages were found in the lateral horn of the grey matter of the lumbo-sacral part of the spinal cord.

The slight focal fatty infiltration of the liver, slight diffuse fibrosis of the lungs, sarcosporidiosis of the myocardium, slight acute lymphadenitis and renal chalicosis should be considered as coincidental and of no importance as far as hydrocyanic acid poisoning is concerned. The haemorrhages in the central nervous system, with the exception of those in the cerebral cortex and thalamus, are very recent and are probably agonal. There is no trace of old haemorrhages. The significance of the changes in the pituitary gland is not clear.

Sheep 52511 was given 1·1 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 15½ months followed by 1·6 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 15 months.

Symptoms.

The ewe died 40 minutes after the last dose of hydrocyanic acid in spite of the administration of sodium thiosulphate. Nothing abnormal had been previously observed in this animal.

Post-mortem Appearances.

General cyanosis; slight hydropericardium; slight hyperaemia of the lungs; slight regressive changes of the myocardium; adhesions between the gall-bladder and mesenterium suspending the small intestine; liver decreased in size with degenerative changes; degeneration of the kidneys, slight tumor splenis.

Histology.

Liver.—Most marked in a zone near the periphery of the lobule, but extending irregularly throughout them, are hepatic cells containing a variable number of mostly large globules of fatty masses. Occasional vacuoles are present in the hepatic cell cytoplasm. The nuclei appear normal. In parts there is a slight to moderate round cell infiltration (i.e. of small lymphocytes and histiocytes) of Glisson's capsule. Small midzonal, or more peripherally situated, foci of lymphocyte and histiocyte (epithelioid and macrophage type) accumulations are present although they are not very frequent.

Kidney.—Autolysis is evident. The mitochondria of the cells of the proximal convoluted tubules are very prominent. Their lipoidal nature is evident with the Sudan III stain. A few fat globules are present in the epithelium of the proximal convoluted tubules. The distal convoluted tubules are affected to a much lesser degree whilst the other tubules are not at all affected.

Adrenal.—A narrow zone in the "*glomerulosa*" contains lipoidal globules of varying size. Some have eccentric vacuoles. The rest of the "*glomerulosa*" just below the capsule is relatively free from sudanophile fats and the *fasciculata* and *reticularis* contain no visibly staining fatty elements. The medula showed no pathological change.

Sheep 52361 was given 1·1 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 4 months.

Symptoms.

The animal died shortly after the last dose of hydrocyanic acid but was not seen at the time of death. Previously nothing abnormal had been observed in the animal.

Post-mortem Appearances.

General cyanosis; hydropericardium; hydrothorax; petechiae in the thymus; hyperaemia and oedema of the lungs; congestion of and regressive changes in the liver and kidneys; very slight hyperaemia of the mucosa of the abomasum, small intestine and caecum.

Histology.

Liver.—There is a moderate but well-defined distention of the sinusoids and of the portal, central and sublobular veins as well as of the branches of the hepatic artery. Around some of the portal tracts slight to moderate lymphocyte and plasma cell infiltration has occurred, these cell types being present in varying proportion. The bile ducts show moderate proliferation. The Kupffer cells are fairly prominent and practically all the endothelial cells lining the sinusoids are swollen. There is oedema

of the hepatic cord—sinusoid interspace. The liver cells are markedly vacuolated either regularly by the presence of small round vacuoles or irregularly by the presence of large rounded or ragged-looking vacuoles. No free sudanophile fat is present. The nuclei of the hepatic cells are normal or slightly swollen whilst occasional ones are crenated. Occasionally polymorphonuclears have penetrated into the hepatic cell cytoplasm. One small focus was observed of polymorphonuclears, plasma cells and lymphocyte infiltration with concomitant endothelial reaction and metamorphosis into histiocytes. There is no increase in connective tissue.

Lung.—The alveoli are more collapsed than normal, such areas of relative atalectasis alternating with emphysematous areas. There is moderate capillary distention and the larger vessels are well distended with blood.

Myocardium.—A few myocardial fibres stain more intensely eosinophilic and a small proportion of these have lost their cross-striation as well as the individuality of the fibrillae, to form a more or less homogeneous eosinophilic mass. The nuclei in these instances have a tendency to become pyknotic. The capillaries are moderately distended.

Spleen.—The sinusoids contain an increased amount of blood. Small lymphocytes are relatively scarce whilst large lymphocytes and plasma cells (both large and small) dominate the picture. Fairly numerous polymorphonuclears are present mostly in the sinusoids but to a lesser extent in the splenic reticulum. The reticulo-endothelial cells are fairly prominent. Here and there a circulating phagocyte containing haemosiderin was observed. Small amounts of haemosiderin around the arteries coursing through the pulpa are indicative of previous minor haemorrhages. Remarkable are the few small irregular foci which show up as uneven eosinophilic areas in which red cells, collagenous tissue, odd specks of chromatin dust and invading phagocytes and young fibroblasts are recognisable. The spleen smear shows a moderate neutrophilia and a predominance of the larger lymphocytic elements.

Kidney.—The capillaries are distended with blood. Fine granules of bile pigment are present in some of the epithelial cells of the proximal convoluted tubules. More sparsely distributed, but in heavier clumps, are haemosiderin masses lying both in the epithelial cells as well as in the endothelial cells of the intertubular capillaries.

Hypophysis.—There is a vascular dilatation of moderate degree, both in the *pars anterior* and *pars posterior* and to a lesser extent in the *pars intermedia.*

Adrenal.—No visible sudanophile fats are present in the cortical cells although here and there a cell stains a diffuse light orange-brown colour with Sudan III. The dark-staining cells in the *reticularis* show marked irregularity of outline and a greater degree of pyknosis than is usually found. They are also present, although singly, in the *fasciculata.* Very similar cells but with slightly less darkly staining and more granular cytoplasm are seen in the "*glomerulosa*". These latter cells are also found in the adrenal *glomerulosa* of normal sheep but are less numerous and by no means show such well-defined eosinophilic staining of the cytoplasm and crenation and pyknosis of the nucleus. This phenomenon was also observed in sheep 52445 but not in sheep 52557. It is most likely a phase in the activity of the adrenal cortex, possibly accentuated in these cases, but not of such a nature that it can undoubtedly be classed as a pathological phenomenon. The medulla revealed no abnormality.

Nervous System.

Cerebrum.—Normal except for a few fresh minor haemorrhages in the white matter.

Thalamus.—Normal.

Midbrain.—Normal except for a scattered number of minor haemorrhages which are very recent. There is no evidence of old haemorrhages.

Cerebellum.—Normal.

Medulla.—A scattered number of minor haemorrhages are present.

Spinal Cord.—Minor haemorrhages are present in the dorsal, lateral and ventral horns.

Femoral Nerve.—Normal.

The haemorrhages in the central nervous system should be considered as agonal.

Sheep 53051 was given 1·1 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 15½ months followed by 1·6 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 10 months. The animal died suddenly overnight without anything abnormal having been observed previously.

Post-mortem Appearances.

Advanced post-mortem changes; general cyanosis; hydrothorax; hydropericardium; tympanites of the rumen; subepicardial petechiae; severe hyperaemia and oedema of the lungs; purulent pneumonia of the cardiac lobe of the right lung; tumor splenis.

Histology.

Microscopical examination of the various organs was not done on account of the advanced post-mortem changes.

Sheep 53203 was given 1·1 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 2 months followed by 1·6 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 17 months.

Symptoms.

The animal died suddenly overnight without anything abnormal having been observed previously.

Post-mortem Appearances.

Advanced post-mortem changes; ascites; hydrothorax; hydropericardium; oedema of the lungs; congestion of the vessels of the brain.

Sheep 59827, 59828, 59473 and 59871 were given 1·6 mg. of hydrocyanic acid per Kg. of bodyweight twice daily for 6½ months without anything unusual being observed. The animals were then discharged from the experiment.

Sheep 52472.—Control ewe. The animal died from acute mastitis 22 months after the commencement of the experiment.

Post-mortem Appearances.

Pronounced general icterus; haemolysis; haemoglobinuria; pronounced pigmentation and degeneration of the liver; acute septic mastitis.

Sheep 52450.—Control ewe. The animal was killed *in extremis* 25 months after commencement of the experiment.

Symptoms.

Four months before death the ewe suffered from dystokia and had to be assisted. Since that time she gradually lost condition and frequently suffered from diarrhoea and anorexia.

Post-mortem Appearances.

Cachexia; severe regressive changes in the liver; petechiae in the adrenals; slight hyperaemia of the abomasum; oedema of the mucosa of the small intestine. Sheep 52340, 52577 and 52534 also served as control ewes. Nothing unusual was observed in them.

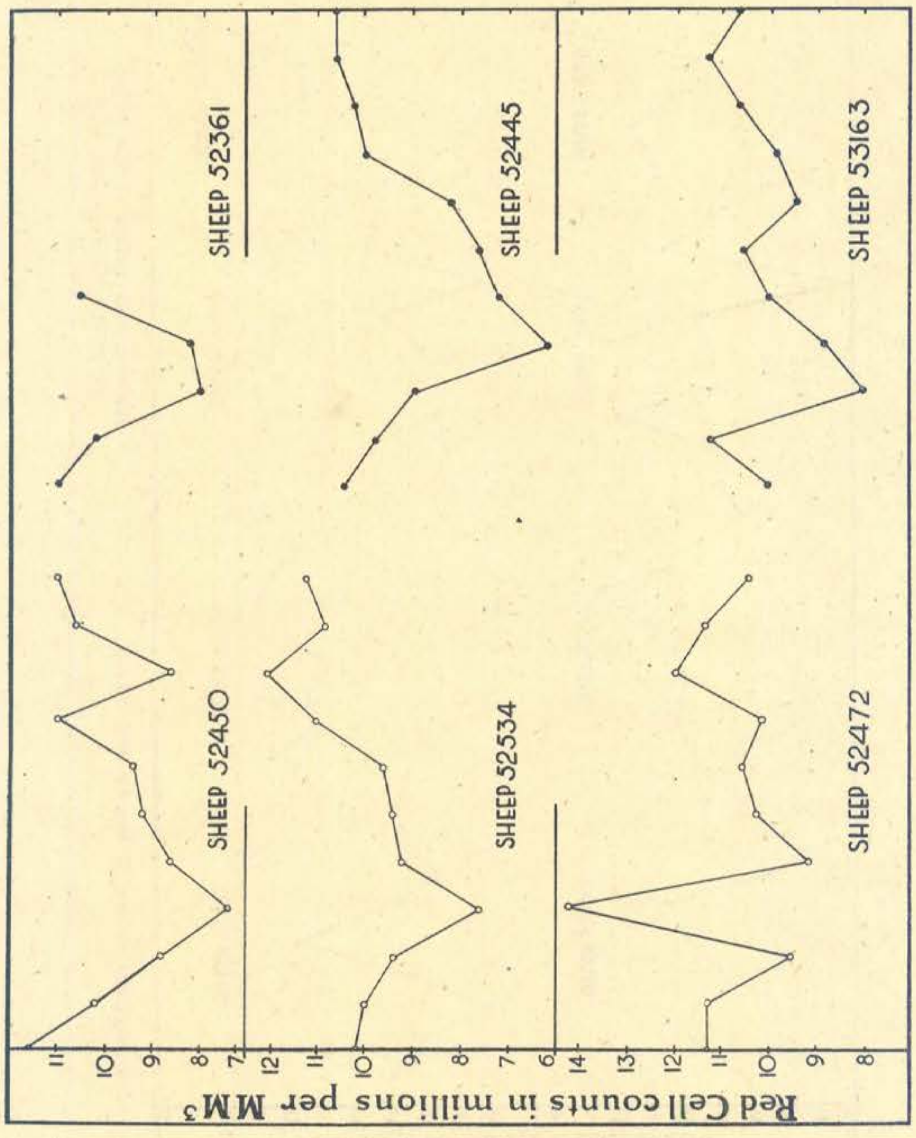
APPENDIX II.

Sheep.	Dates on which the Sheep Exhibited Extremely Severe Symptoms of Hydrocyanic Acid Poisoning.																	
52445	9/3/40	* 3/4/40	11/4/40	* 13/5/40	* 5/6/40	* 11/11/40	4/ 1/41	10/2/41	1/3/41	* 18/3/41	19/3/41	4/4/41	* 9/4/41	* 12/6/41	15/7/41	16/7/41	6/8/41	22/8/41
53203	14/2/40	* 13/3/40	12/4/40	9/6/40	* 22/8/40	* 23/10/40	13/12/40	17/1/41	20/2/41	12/3/41	* 18/3/41	19/3/41	1/4/41	* 23/5/41	23/5/41	4/6/41	† —	—
52551	21/1/40	27/2/40	* 4/3/40	9/3/40	* 3/5/40	* 15/7/40	* 6/11/40	9/1/41	12/2/41	* 18/3/41	19/3/41	24/3/41	29/3/41	* 1/4/41	3/4/41	7/4/41	* 9/4/41†	—
53051	23/3/40	* 11/7/40	13/8/40	* 21/10/40	* 6/11/40†	* —	—	—	—	—	—	—	—	—	—	—	—	—
53163	2/3/40	* 3/4/40	9/3/40	* 10/3/40	* 11/4/40†	—	—	—	—	—	—	—	—	—	—	—	—	—
59827	18/3/41	19/3/41	* 18/6/41	19/6/41	—	—	—	—	—	—	—	—	—	—	—	—	—	—
59828	* 18/3/41	19/3/41	* 4/4/41	* 9/4/41	* 13/6/41	* 18/ 4/41	19/ 6/41	15/7/41	6/8/41	16/8/41	18/8/41	22/8/41	—	—	—	—	—	—
59473	18/3/41	* 19/3/41	24/3/41	* 12/6/41	* 13/6/41	16/ 6/41	23/ 7/41	* 27/4/41	28/7/41	6/8/41	2/8/41	* 22/8/41	* 29/8/41	—	—	—	—	—
59871	18/3/41	24/3/41	29/3/41	* 16/6/41	* 6/8/41	—	—	—	—	—	—	—	—	—	—	—	—	—

This record was not kept from the beginning of the experiment but only from the time (8/1/1940) the dose of hydrocyanic acid was increased from 1·1 mg. to 1·6 mg. of hydrocyanic acid per Kg. of bodyweight. On those days, marked with an asterisk, the sheep had to be treated with sodium thiosulphate to prevent death from occurring.

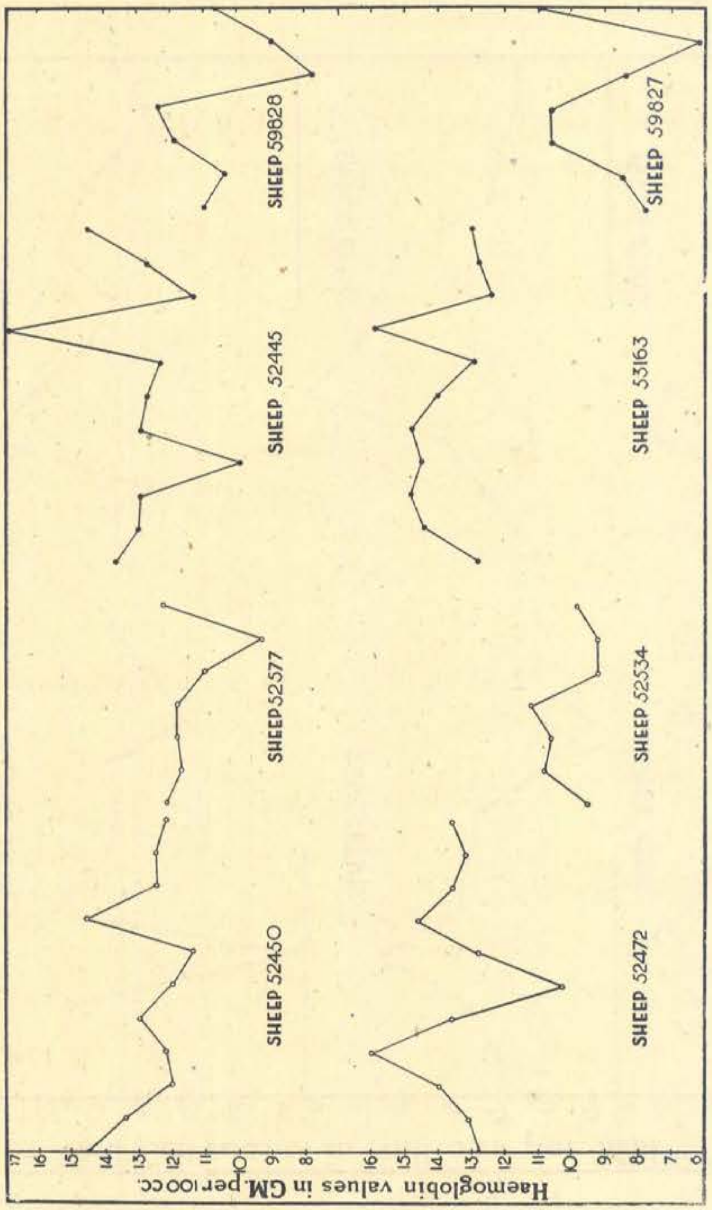
† = Exitus l talis.

APPENDIX III.



Red Cell Counts of the Control Sheep.

Red Cell Counts of the Sheep receiving Hydrocyanic Acid.



Haemoglobin values of the Control Sheep.

Haemoglobin values of the Sheep receiving Hydrocyanic Acid