# THE COLLECTION OF TOXIC GRASS AND THE PRESERVATION OF TOXICITY

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THE RECOGNITION (Hopkirk, 1936) that facial eczema involves not eczema but photosensitization, and that the essential lesions are not on the face but in the liver, stressed the analogy of this disease to poisoning by *Tribulus, Lippia* and *Lantana* previously investigated by South African workers. This analogy, together with the failure of all bacteriological transmission experiments, led to the hypothesis that the disease was caused by a toxic substance in autumn flush pastures. The testing of this hypothesis by feeding suspected grass under controlled conditions was rendered difficult by the sporadic and unpredictable occurrence of the disease and by the delay between ingestion of toxic grass and appearance of symptoms. It was not until 1941 that Filmer and co-workers first produced typical liver lesions in lambs fed in pens on green grass cut from a field in which grazing lambs developed the disease (Simpson *et al.*, 1957).

This experiment did not strictly reproduce the complete facial eczema syndrome by experimental feeding, since that requires production of icterus and photosensitivity in addition to liver damage; in fact the first production of icterus in lambs fed dried grass was achieved only 2 months ago by J. C. Percival, and the complete syndrome including photosensitization has not yet been demonstrated. Filmer's experiment, however, marks the starting point of all subsequent work on the isolation of the liver damaging principle. It also provided a rational basis for systems of control based on removal of sheep from potentially toxic pasture.

Before isolation of the liver poison could be pursued two further steps were necessary:

(1) The devising of a system of detecting when grass was toxic.
(2) Provision of a method of preserving the grass with as little loss of toxicity as possible.

## Determination of Toxic Period

From the outset, because the grass remained toxic for a comparatively short time, and because the toxin could be detected only through animal feeding, it was apparent that grass must be mown and preserved "on spec" pending evidence of when it was

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toxic in the paddock. To define the period of toxicity the following procedure was devised by Filmer. (The description refers to the system actually in use at Manutuke over recent years, Simpson *et al.*, 1957).

Early in January lambs are brought from an area free from facial eczema and placed on crops (turnips and thousand-headed kale) which are known to be safe. Before the first autumn rains a group of about 40 of these lambs is placed on the collection area. This consists of ten acres of perennial ryegrass/white clover, subdivided into five 2-acre paddocks. The lambs are rotationally grazed, normally spending two days in each paddock. After three weeks, and weekly thereafter, two of these lambs are removed. One is killed immediately for liver examination, the other is kept on crop for three weeks before killing.

Each week ten further lambs are introduced to this area and similarly rotated for three weeks, after which five are killed immediately and five held on crop for three more weeks. (The purpose of holding some lambs before killing is to allow further development of the liver lesions which have been found to progress after removal from the toxic feed.) At killing the lamb livers are examined for facial eczema lesions. Any clinical eczema in the groups is also recorded. In this way a series of groups of lambs are obtained. Some have been on the collection area for varying periods extending from the start of the season, others for overlapping three-week periods. Appearance of liver damage in any of the groups indicates that grass was toxic at some time in that grazing period. By comparing overlapping periods, and taking into account the degree of toxicity as judged by number affected and severity of lesions, the toxic period can be approximately defined.

### Collection of Grass

While the toxic period is being determined in this manner, to ensure that toxic grass is collected it is necessary to mow frequently, preferably each day, some of the area on which the indicator lambs are grazing.

### Preservation of Toxicity

For preservation of toxicity in the grass the range of methods likely to be practicable has been limited by the large amounts to be handled. In early work several methods of limited application were tried, including canning of expressed juice, and of grass boiled in water, and pickling in alcohol. None of these were

satisfactory. Fortunately the most practicable method, rapid drying in a blast of hot air, produced in 1943 dried grass sufficiently toxic to cause severe liver lesions when fed to lambs for 36 days. Since that time almost all the toxic grass collected has been preserved by rapid drying.

In the drying procedure air is heated by coke or oil burners and blown through a 6 to 8 in. layer of grass packed in bins. The air entering the grass mass is controlled as closely as possible around 100 to 105°C. The hot air is passed through one set of bins for 10 min, then deflected to the second set for 10 min while grass in the first is turned and teased. Usually three 10 min spells are required, so that grass is in the bins about one hour altogether, but the time required depends on the state of the grass.

Since this procedure has been criticized from time to time it is worth pointing out that it was originally introduced with some scientific basis, namely that rapid heat drying might inactivate destructive enzymes; and that dried grass appeared the most convenient form for storage, test feeding and subsequent chemical manipulation. Later work has indicated that drying would have been inevitable at some stage before extraction. It is not claimed that heat drying does not involve some loss of toxicity. At one stage it appeared the most likely reason for failure to secure toxic grass when lambs grazing the paddocks had developed clinical facial eczema. On the other hand sampling differences between material consumed by sheep and that collected by mowers may have accounted for this failure. Unfortunately, because of problems in inducing sheep to eat mown green grass, and in predicting toxicity, as well as variation in animal sensitivity, the toxicity of freshly cut grass has never been adequately compared against dried grass.

### Use of Guinea Pig in Testing Grass

In the ten years 1943-53 toxic grass in varying amounts was collected at Manutuke in four years. Most of this grass was used in test feeding trials and in preliminary large scale extraction experiments using sheep as test animals. With the development of the guinea pig assay of toxicity, the much smaller amounts required for this test suggested a new approach to definition of the toxic period, as indicated by preserved grass. Previously, to secure an adequate bulk for sheep feeding, it had been necessary to combine all cuttings from one area over the period indicated as toxic by the grazing sheep. This introduced the hazard that some

highly toxic grass cut on one or a few days might be diluted with less toxic or non-toxic grass on other days within the estimated toxic period. When guinea pigs are used to check toxicity of the grass, bulking is not necessary and each day's collection can be tested separately. This procedure was introduced by the writer to test a series of small samples collected at Claudelands Showgrounds in 1953 during a suspected toxic period, without the use of grazing sheep. In subsequent years larger collections were made in this way in the Waikato, providing some of the most valuable grass used in the isolation work. Although the use of grazing sheep has been continued at Manutuke Station, since 1953 the testing of individual daily samples with guinea pigs has been the standard method of checking the toxicity of dried grass.

Guinea pig testing is the more satisfactory means of checking toxicity of dried grass, particularly as most subsequent use of this grass involves guinea pig feeding, but reservations must be made about interpreting such results in terms of toxicity in the field, since mown material is not the same as that grazed by sheep.

As requirements for toxic grass increased, the collection programme was extended. A design which had as its main object the inclusion of more cutting areas, to increase the chance of success if outbreaks were localized, was initiated by Ruakura staff in 1956, and put into effect, with increased facilities, in 1957. In that year the collection team, supervised by Percival, mowed regularly eleven areas in the Waikato. Here little facial eczema occurred, and of the 450 samples, amounting to 8 ton of dried grass, only about 1 cwt was toxic. At Gisborne, Sinclair had more success. From four farms in addition to Manutuke he collected 5½ ton, of which 1¾ ton was toxic in varying degree. On the other hand, all of the toxic grass obtained in the same way in 1958 (on present estimate about 1 ton) came from the Waikato areas.

### Use of Beaker Test

A further step in the grass collection programme was the introduction of the "beaker test". This test will be described and its value assessed later in this symposium. Here it is sufficient to state that it is a test which indicates, within a few hours, whether grass is likely to be toxic. Pasture giving a negative test has rarely proved toxic; pasture giving a strong positive test, early in the season, is sufficiently likely to be toxic to justify collection. The reliability appears to fall considerably after the first period of toxicity.

This test has been used in the grass collection programme over the past two years as a guide for when and where to cut large amounts for drying.

### Storage of Dried Grass

Originally dried grass was stored in sacks or wool bales. On several occasions samples kept under dry conditions in this way have been found to lose toxicity. To reduce such loss the grass is now packed, without milling, direct from the dryer into milk powder tins, and these are held in cold storage until required.

### Preservation by Freezing and Freeze Drying

As mentioned earlier there is little information about loss of toxicity during cutting and drying. In several years no toxic grass, or very little, has been obtained at Manutuke although lambs on the collection areas had shown quite severe damage. For example, in 1956 only two samples (both cut on the same day, but from different areas) were toxic. The lamb killing data indicated that grass was toxic over a period of at least three weeks.

Such results cast some suspicion on the drying procedure. As an alternative, storage of the grass in a frozen state, with subsequent freeze drying, has been investigated. During 1957 and 1958 a number of samples were divided into two portions, one being heat dried immediately and the other frozen quickly to —20°C and held at this temperature. Later the frozen samples were dried in a tray-type freeze dryer with condenser set at —40°C. For most batches the temperature was about —8 to —10°C at the start of drying, rising eventually to room temperature.

In 1957, of 28 samples preserved by freezing, only one proved toxic. Both the heat dried and the freeze dried material produced mild liver damage. Of samples frozen in 1958 three were toxic, as indicated by the heat dried portion. Feeding tests are not yet completed, but results again do not indicate any difference between heat dried and freeze dried portions beyond what can be expected from animal variation.

These experiments have established that toxicity at the "severe" level can be retained over a period of 6 months by rapid freezing and subsequent holding at —20°C.

Toxic grass will be needed for some purposes for some time yet, but complete dependence on it has been removed by recent

developments incriminating *Sporidesmium* as the toxin producer. Probably few appreciate this fact more than those people (among whom should be mentioned Messrs. Simpson, Sinclair and Percival) who have spent much time and effort in the often unrewarding job of collecting and preserving toxic grass.

## Literature Cited

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