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# THE BEAKER TEST AS AN INDICATOR OF FACIAL ECZEMA TOXICITY

N. T. CLARE\*

DURING THE FRACTIONATION of extracts of toxic grass E. P. White isolated a substance which appeared as a white film on glassware as solvents were evaporated (White, 1958). Similar fractions from non-toxic grass did not yield this material. It was soon established that this substance could not, itself, be the facial eczema poison, but the apparently close association of the two suggested a means of distinguishing between toxic and non-toxic samples. Dr D. D. Perrin adapted White's extraction and fractionation procedures to develop a technique for detecting this substance in 50 g samples of dried grass. From a survey of all toxic samples available in 1956, as well as a number of non-toxic samples from various sources, Perrin confirmed an association between the presence of this substance and toxicity. Because the final detection of the white material depended on deposition on the side of a beaker, he applied the name "beaker test" (Perrin, 1959).

During the 1957 season Perrin's test was applied to a large series of samples, collected around Gisborne and the Waikato, which were also tested for toxicity by guinea pig feeding. Subsequently the procedure for detecting the beaker test substance was considerably modified (Sandos *et al.*, 1959), the time required being reduced so that the test could be completed within 4 to 6 hours of receiving the sample instead of 36 hours. This made it possible to cut a sample, beaker test it, and, if the test was positive, collect a larger amount of grass from the same area next day, and frequently within the same day. In addition, the new procedure eliminated interference by pennyroyal, a defect which rendered the original test useless on many Waikato pastures.

The test in its present form is carried out as follows: A 50 g sample of ground grass is extracted by percolation with 100 ml of acetone. The acetone extract is passed down a two piece chromatogram, the upper tube containing alumina, the lower an adsorptive carbon. Further acetone is run in under pressure. The first 75 ml of eluate is discarded, the second 75 ml portion is evaporated before a fan, when a positive test is indicated by a white deposit on the beaker wall. Positive tests are graded

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according to the amount of the deposit, comparison being made against arbitrary standards prepared from a series of pastures giving different levels.

The substance responsible for the test is chemically very inert, and no distinctive colour reactions or other properties suitable for its detection or estimation have been found. In the meantime the rather unsatisfactory method of detection and grading must be used. Lack of a satisfactory end estimation probably led to errors in assessment of early tests with consequences which will be mentioned later.

As White has reported in this symposium, the beaker test substance is now known to be a constituent of the fungus *Sporidesmium bakeri* which also produces the facial eczema toxin. This explains why beaker test and toxicity are commonly associated; but it does not mean that such an association is invariable. The beaker test is primarily an indicator of the presence of the fungus (probably of the spores specifically) rather than a criterion of toxicity.

### Comparison of Beaker Test Against Toxicity

In the first comparison of beaker test against guinea pig toxicity made by Perrin on samples cut in 1955 and 1956, 12 out of 13 samples containing the toxin gave a positive beaker test and there was a rough parallel between the level of the test and severity of liver lesions. In 1957 Dr Perrin and Mrs Sandos applied the test to 393 samples from the Waikato and Gisborne. As no facial eczema outbreak occurred in the Waikato areas, the samples from this district will not be considered at this stage. An analysis of results on the 228 samples from Gisborne is given in Table 1.

Apart from the fact that over 75 per cent. of the toxic samples were also beaker test positive, consideration of all the samples indicates that a positive test is not much better than coin tossing as a prediction of toxicity.

TABLE 1: BEAKER TEST (B.T.): GUINEA PIG (G.P.)  
RELATIONSHIP, 1957

<i>All Samples</i>							
	B.T. +	B.T. -	G.P. +	G.P. -			
B.T. +	.....	.....	65	69			
B.T. -	.....	.....	19	75			
<i>Fed Within 14 Weeks</i>				<i>Fed After 14 Weeks or More</i>			
	G.P. +	G.P. -		G.P. +	G.P. -		
B.T. +	.....	53	17	B.T. +	.....	12	52
B.T. -	.....	17	29	B.T. -	.....	2	46

### EFFECT OF LOSS OF TOXICITY

However, during the guinea pig feeding trials, which, because of the numbers, extended over several months, results were obtained indicating that many samples may have lost toxicity before they were fed. For this reason the samples were resolved into two groups, those fed within 14 weeks of cutting, and those fed after this period. Results of this subdivision appear in the second portion of Table 1.

It is apparent that for the samples fed within 14 weeks, and thus less likely to have declined in toxicity, there is a much closer association between beaker test and toxicity.

### EFFECT OF TIME OF CUTTING

During this work it became apparent also that beaker tests had continued beyond the likely toxic period as indicated not only by previous concepts of weather and growth conditions, but also by liver damage in the grazing lambs. No lesions occurred in lambs introduced into the Manutuke experimental areas after 25 March; yet beaker tests were recorded on samples cut as late as 16 April. Accordingly the association between beaker test and toxicity was further examined by grouping the samples by date of cutting—those cut up to 25 March, and those cut after this date. This analysis, for “fed within 14 weeks” samples only is given in Table 2.

These results indicate that pastures may continue to give positive beaker tests after they have ceased to be toxic.

### SAMPLES NEGATIVE BY BEAKER TEST, BUT GUINEA PIG POSITIVE

A disturbing feature of the results shown in these tables is the number of samples which were negative by the beaker test, but toxic when fed. Of the 84 toxic samples shown in Table 1, 19 gave no beaker test, but when these 19 were retested, 17 were recorded as positive. This discrepancy appears to have arisen through difficulties with standards in the early period of testing, and there is no doubt that the repeat tests are correct. Because

TABLE 2: ANALYSIS BY CUTTING DATE

<i>Fed within 14 weeks</i>			
<i>Cut up to 25 March</i>		<i>Cut after 25 March</i>	
		G.P. +	G.P. —
B.T. +	.....	53	17
B.T. —	.....	17	29
<i>Cut up to 25 March</i>		<i>Cut after 25 March</i>	
B.T. +	.....	0	13
B.T. —	.....	0	4

most of the non-toxic beaker test negative samples had already been discarded and could not similarly be retested, only the first tests have been used in the comparison tables. It is fair enough, however, to state that of the total of 84 toxic samples cut in 1957 only two consistently gave negative beaker tests.

The conclusions from the 1957 tests may be summarized as follows:

- (1) For samples collected during the toxic period and fed without the delay which may have caused decline in toxicity, there is a close association between beaker test and toxicity.
- (2) The beaker test is not reliable later in the season, the substance responsible for it being apparently retained in pasture after the toxin has disappeared.
- (3) Samples which do not give a beaker test are unlikely to be toxic.
- (4) For practical purposes the test is useful for the selection early in the season of suitable areas for grass collection and field experiments.

#### SAMPLES COLLECTED IN 1958

From the samples collected in 1958 in the Waikato, a different picture has emerged. In this year there is no evidence of complications due to possible loss of toxicity during storage, all samples being refrigerated until used. An analysis made by J. C. Percival is given in Table 3.

The main differences between these results and those for 1957 is the appearance of beaker tests before toxicity, and the high proportion (19 per cent.) of samples negative by beaker test but positive by guinea pig. Unfortunately, regular collection of samples for test feeding ceased on 19 March, so that the length of the toxic period cannot be defined and the question of continuation of beaker tests beyond toxicity cannot be examined.

TABLE 3: WAIKATO SAMPLES, 1958  
(Toxicity extended from 2 March to at least 19 March)

<i>All Samples—18 February to 3 April</i>			
	G.P. +	G.P. —	
B.T. +	....	....	22
B.T. —	....	....	5
			28
			74
<i>Samples Cut 18 to 28 February</i>		<i>Samples Cut 1 March to 3 April</i>	
	G.P. +	G.P. —	
B.T. +	....	0	15
B.T. —	....	0	25
			22
			13
			5
			49

Three possible explanations of the main difference between the 1957 and 1958 results, namely the occurrence of positive beaker tests before toxicity, are suggested.

- (1) In 1957 comparatively few samples were collected before toxicity developed. If more had been available it is possible that some beaker tests might have been recorded during the build up of *Sporidesmium* to a dangerous level.
- (2) In 1957, the grass samples were ground before feeding. In 1958 they were fed without grinding—a procedure considered then to be more satisfactory because the guinea pig ate the green material and rejected dried stalk and debris. However, with samples in which most of the fungus was on the litter, such a feeding technique may have failed to detect toxicity, although beaker tests done on the ground material were positive.
- (3) Some of the beaker test positive samples may have contained the poison, but at a level not detected by direct feeding to guinea pigs. In his first comparisons Perrin found that some beaker test positive samples, which produced no lesions by direct feeding, gave extracts which were toxic when fed at a higher level. It is of interest that some of the areas which gave beaker test positive, but non-toxic, samples early in 1958, later became toxic.

While these possibilities are suggested to explain the difference between the 1957 and 1958 results, it must be queried whether it is necessary to seek such explanations in terms of techniques. After all, little is really known about the relation between the hepatotoxin and the beaker test material, beyond the fact that both are produced by *Sporidesmium*. It may not always produce both at the same time or in fixed proportions. It may not be the only microorganism which will give a positive beaker test. Nothing is known of the metabolic or chemical relationships between the two substances, and their association may depend on strain, stage of growth or substrate of the fungus. The beaker test substance has often enough been found in pastures when the toxin is absent. This may be due to alteration of the toxin, which appears the less stable chemically, but it may well be due to other factors. Similarly, from our present knowledge, it cannot be assumed that the toxin is never present when the beaker test substance is absent.

### Limitation of Beaker Test

It is obvious that the beaker test in its present state has serious limitations as an indicator of toxicity. It is, however, reasonable to consider its usefulness for the purpose for which it was originally introduced—that is, as a guide for the collection of toxic grass. If collection had been based entirely on beaker tests in the Waikato in 1957, only six large scale mowings would have been made instead of 165, and two of the six would have been toxic, while none would have been missed. At Gisborne, on the basis of the samples fed within 14 weeks, 70 would have been collected instead of 116, yielding 53 toxic samples.

In 1958 no large scale collection would have been made at Gisborne, and the data from grazing lambs indicated that there was no toxicity. In the Waikato, although 50 samples would have been collected to give 22 which were toxic, mowing of 74 non-toxic samples would have been avoided, and only 5 toxic samples would have been missed. Yet this is not the whole story. The bulk of toxic grass for 1958 was obtained two weeks after the dangerous rainfall from an area which was not available earlier. If this area had not then shown higher beaker tests, such large scale collection would never have been made at this time.

In conclusion, even if the beaker test is superseded tomorrow by a better indicator of toxicity in pasture, it deserves an honourable place in the annals of facial eczema research for the part it played, as Percival in this symposium will indicate, in incriminating *Sporidesmium*.

### Literature Cited

- PERRIN, D. D. (1959): *N.Z. J. Agric. Res.*, 2: 266.  
SANDOS, J., CLARE, N. T., WHITE, E. P. (1959): *ibid.* (in press).  
WHITE, E. P. (1958): *ibid.* 1: 859.

### DISCUSSION

DR G. W. BUTLER: Mr Clare has summarized very fully the merits and limitations of the beaker test procedure developed by White and Perrin. It may be of value to consider his findings along with those obtained at Palmerston North since we employed a slightly different experimental approach and also used sheep for our toxicity testing instead of mowing the grass and feeding it to guinea pigs.

A localized incidence of facial eczema on four pastures of perennial ryegrass (paddock 16, Massey College) in autumn, 1958, enabled some observations to be made on the value of the beaker test. These were reported in a preliminary fashion at the Massey Sheepfarmers' Conference, 1958 (*Sheepfarming Annual 1958*, p. 203), but additional data have since

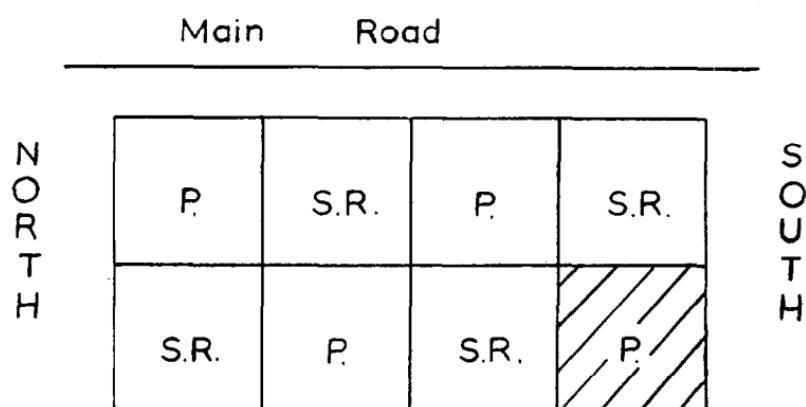


Fig. 1: Layout of perennial (P.) and short-rotation (S.R.) ryegrass blocks on paddock 16 of Massey College sheep farm.

come to hand following the slaughtering of all the experimental animals. The experimental results will therefore be summarized here.

The layout of the four perennial paddocks is shown in Fig. 1, each paddock being 1.2 acres in area. Facial eczema warnings were issued over the period 18 to 26 February but throughout February and early March, composite samples of grass taken from the four paddocks by a random sampling procedure gave negative beaker tests. On 12 March, however, facial lesions and photosensitivity were observed with several sheep grazing one paddock (shaded-in portion Fig. 1) and beaker tests were strongly positive on grass taken from this paddock, but negative on grass taken from the other three paddocks. The fluctuations in beaker tests from grass

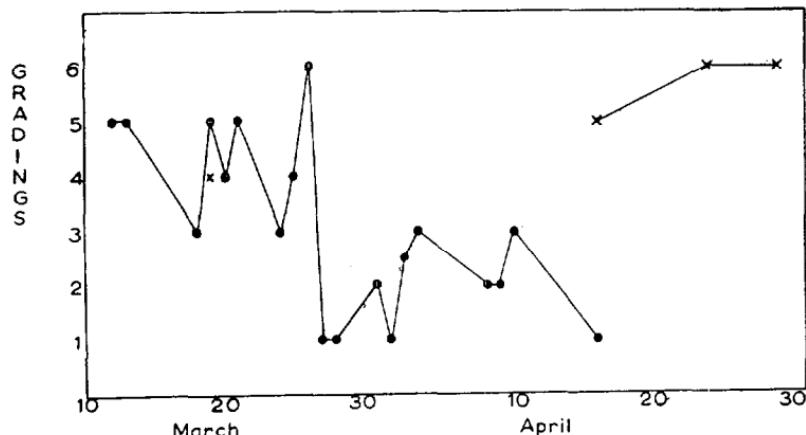


Fig. 2: Beaker tests made on mixed green and dead herbage (circles) and on dead leaves alone (crosses).

collected from the paddock where clinical cases occurred are shown in Fig. 2. It was observed that the beaker test continued high on dead leaf litter from this paddock during April, but tests on leaf litter from the other three paddocks were negative.

Dr R. H. Thornton received samples of grass from the toxic paddock and observed very high spore counts for *Sporidesmium bakeri*. The relative concentration of spores in the three samples was: 13 March—21; 19 March—16.5; 27 March—1.

From the subsequent slaughtering of the experimental animals, the following information about distribution of toxicity was obtained:

- (1) All paddocks were toxic during the latter half of February, with the south paddock giving the most severe liver damage.
- (2) Subsequent to 13 March, there was a gradation in toxicity from south to north, with the south paddock still giving much more severe liver damage.

The details of liver damage (based on microscopic observations) are set out in Table 1.

Thus, although the beaker tests revealed the presence of *Sporidesmium bakeri* on the most toxic paddock, they were negative on the other three paddocks, where considerable liver damage occurred.

TABLE 1: LIVER DAMAGE OF SHEEP GRAZING PERENNIAL RYEGRASS DURING AUTUMN, 1958

Damage Prior to 12 March					
		Nil	Slight	Moderate	Severe
Paddock 1	....	....	1	1	—
3	....	....	4	—	1
6	....	....	—	4	4
8	....	....	1	1	—
Damage After 12 March					
Paddock 1	....	....	2	—	—
3	....	....	1	—	—
6	....	....	—	1	1
8	....	....	—	—	2

L. HARTMAN: In the Fats Research Laboratory we have had the opportunity of investigating the fungal culture and we found that the beaker test substance occurred again and again during our chemical processing. We had the impression that the beaker test substance was being created during this procedure.

N. T. CLARE: We have not made these observations although some made by Dr D. Russell did suggest that Mr Hartman's findings could be correct. I also believe that I. R. C. McDonald of the Dominion Laboratory is strongly in favour of this idea.