THE DETECTION OF RICIN

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The high toxicity of castor seed makes its detection in feeding stuffs a matter of some importance, and the fact that there is no specific chemical test for ricin, its toxic principle, renders this detection extremely difficult. Hitherto no satisfactory method has been described.

Microscopic examination which was investigated by Leather[1] and Dodd[2] is unsatisfactory, because as Brioux and Guerbet[3] point out, it is only the non-toxic testa that can be identified and this will be present in boiled non-poisonous castor meal, but not in the highly toxic decorticated seeds.

The agglutination test, recommended by Autenrieth[4] has been investigated by many workers, including Miessner and Rewald[5] and Kobert[6], the latter claiming to be able to detect 0·2 per cent. of castor seed in meal by this method. Lander and Geake[7] were unable to detect less than 10 per cent. of castor seed in linseed by agglutination. The chief disadvantage of the method lies in its non-specificity as many plants contain non-toxic agglutinins (Mendel[8]).

Miessner[9] suggested that use should be made of the precipitin reaction discovered by Jacoby[10]. This method is also advocated by Bamford[11]. It is claimed to be quite specific but is unfortunately insufficiently delicate to be of practical value, as Lander and Geake[7] were unable by this means to identify ricin in the omasum of an experimentally poisoned calf.

Clarke[12] suggested that use should be made of the fact that serum from an immune animal will neutralise the toxicity of ricin. This property is quite specific (Ehrlich[13]). Details of this method are now given.

Experimental

10 g. of the material to be tested is finely ground and extracted with 50 ml. of 0·02N hydrochloric acid for several hours with mechanical stirring. The mixture is then centrifuged, the supernatant liquid decanted and the residue re-extracted with a further 50 ml. of 0·02N hydrochloric acid, and again centrifuged. The two extracts are combined and filtered if necessary through sintered glass. 300 ml. of acetone is now added to the extract. The precipitate is centrifuged off, transferred to a sintered glass crucible, and dried as far as possible at the pump. It is then extracted with 5 ml. of physiological saline solution and filtered.

The filtrate is divided into two parts, and from each a series of dilutions is prepared; the resulting two identical series of solutions (A and B) represent 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc., of the concentration of the original extract. 0·1 ml. of normal serum is added to each tube of series A, and 0·1 ml. of serum from an immunised animal (goat or rabbit) to each tube of series B. Both series are then injected into mice (0·5 ml. intraperitoneally). If the mice in series A show an appreciably greater mortality than those in series B, the original material must have contained ricin, as the immune serum will protect against no other substance.

Should all mice in both series die the experiment must be repeated

using greater dilutions, in order to decide whether the deaths are due to some other poison, or to the fact that the quantity of ricin present is too great to be neutralised by the serum.

Table I shows the results obtained using cattle cake to which 0·1 per cent. of ground castor seed has been added.

TABLE I

Tube	Dilution	Series A (+ normal serum) Number of mice	Series A (+ normal serum) Result	Series B (+ anti-ricin serum) Number of mice	Series B (+ anti-ricin serum) Result
1	1	3	x 24 hrs., x 24 hrs., x 48 hrs.	3	s, s, s
2	½	3	x 24 hrs., x 24 hrs., x 72 hrs.	3	s, s, s
3	¼	3	x 48 hrs., x 48 hrs., x 96 hrs.	3	s, s, s
4	⅛	3	x 48 hrs., x 48 hrs., x 72 hrs.	3	s, s, s
5	1/16	3	x 72 hrs., x 72 hrs., x 72 hrs.	3	s, s, s
		15		15	

x = died. s = survived.

TABLE II

Tube	Dilution	Series A (+ normal serum) Number of mice	Series A (+ normal serum) Result	Series B (+ anti-ricin serum) Number of mice	Series B (+ anti-ricin serum) Result
1	1	2	x 36 hrs., x 36 hrs.	2	s, s
2	½	2	x 60 hrs., s	2	s, s
3	¼	2	s, s	2	s, s
4	⅛	2	s, s	2	s, s
5	1/16	2	s, s	2	s, s
		10		10	

x = died. s = survived.

Table II shows the results obtained in an experiment in which 0·005 per cent. of castor seed was added to cattle cake. This represents 1 p.p.m. of ricin, which is the smallest quantity that can be detected by this method.

Similar results were obtained using castor seed added to sunflower seed, field beans, soya beans and toppings. With linseed cake the method is not so delicate, the limit being 0·1 per cent. of castor seed.

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