PRODUCTION OF IgGs TO RICININE FROM THE CASTOR-OIL PLANT

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Castor-oil plant meal, which is a potential full-value protein-rich fodder, may, depending on the technological sheme adopted, contain various levels of the toxic components that are present in the native seeds of the castor-oil plant – the proteins agglutinin and ricin, the allergen CB-1A, and the alkaloid ricinine.

The alkaloid ricinine is a monocyclic pyridine derivative with cyano and methoxy groups and has the formula $C_8H_8O_2N_2$. Its melting point is 201.5°C and its molecular mass 164.0 (by mass spectrometry) [1].

The aim of the present investigation was to obtain IgGs from rabbit antiserum for the development of an enzyme-linked immunometric method of determining ricinine quantitatively in the seeds of the castor-oil plant and the products of its processing.

The low-molecular-mass substances, including ricinine, are nonimmunogenic and cannot themselves induce antibody formation [2]. However, antibodies to low-molecular-mass compounds can be obtained by injecting into an animal an artificially conjugated antigen – a hapten covalently bound to an immunogenic carrier.

Ricinine was isolated from the defatted flour of the seeds or meal with 85% aqueous ethanol. The extract was concentrated and was made alkaline with ammonia solution, and was reextracted repeatedly with chloroform. The completeness of extraction was monitored by the TLC method on Silufol in the UV at 254 nm. The chloroform extract was distilled to dryness, and the residue was crystallized from ethanol.

To prepare the hapten, we selected the optimum conditions for obtaining "ricininic acid." The hapten was conjugated with bovine serum albumin with the aid of a water-soluble carbodiimide. The resulting conjugate was dialyzed against distilled water, freeze-dried, and stored in the cold until use. We have developed a rabbit-immunizing scheme for obtaining antibodies to the conjugate. Immunization was carried out subcutaneously at 30 points in the region of the neck and spine using as the immunogen an emulsion of the conjugate in 0.14 N NaCl with complete Freund's adjuvant (1:1). The fraction of IgG immunoglobulins from the serum so obtained was isolated by precipitation with ammonium sulfate and gel fibration on DEAE-Sephadex A-50 [3].

This communication forms part of a scientific investigation in which the immunoglobulins isolated, subsequently linked with peroxidase, will play the role of labeled antibodies to be used in the method under development.

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