THE QUANTITATIVE DETERMINATION OF SOY-BEAN PROTEIN IN SAUSAGE OR OTHER PROTEIN MIXTURES

GOVERNMENT restrictions forbidding the use of soy-bean flour in sausage or other meat used in interstate commerce is based on the lack of a reliable test for the quantitative determination of soy-bean protein in such meat. The nutritional value of soy-bean is not questioned. At the present time no strictly chemical method of assay has proven reliable.

We have recently obtained accurate quantitative results by the use of an immunological method which is both simple and rapid. The method is based on a quantitative precipitin test, the "optimal proportions" reaction, first described by Dean and Webb[1] and subsequently proven by Taylor, Adair and Adair[2] to be well within the limits of accuracy of the best-known chemical methods.

The test depends on the fact that in any antigen-antibody titration system the velocity of the reaction is related to the proportion of antigen to antibody. Thus, for a given antibody, precipitation is most rapid when the ratio of antigen to antibody is at an optimum which can be readily determined. This optimum ratio is a constant for each antibody solution and is independent of the concentrations of either antigen or antibody in any specific test.

For example, suppose a given antigen reacts most rapidly with a given antibody at a ratio of 1 to 50; that is, one part of antigen forms a precipitate with 50 parts of antibody at a faster rate than with 45 or 55 parts of antibody. In fact, any ratio other than 1 to 50 will be slower than this optimum. Then, since this optimum ratio is a constant, the actual concentration of reagents may vary within fairly wide limits. It may be 3 to 150 or 10 to 500 or 25 to 1,250.

It is a simple matter to standardize any particular antibody against a known antigen in terms of optimal ratio. Using this ratio the concentration of antigen in any unknown mixture can be determined.

In the specific instance of quantitative assay of soy-bean protein in sausage, the test is performed as follows:

Rabbits are immunized against a 5 per cent. NaCl extract of soy-bean flour. For practical purposes, it is unnecessary to use purified glycinin. Several courses of injections over three or four months are usually necessary to produce a serum of satisfactory potency. The serum is collected and standardized against known soy-bean flour extract. Its optimal ratio is determined as accurately as possible. This ratio is then a constant for that particular serum.

Sausage containing soy-bean flour is extracted with 5 per cent. NaCl. This unknown extract is then titrated against the standard serum and its ratio determined. By dividing the test ratio by the standard ratio the percentage of soy-bean protein in sausage is given.

For example, a standardized serum had a ratio of 1 to 30 against pure soy-bean flour extract. An extract of sausage gave a ratio of 1 to 3 with this serum. Therefore the sausage contained 10 per cent. of soy-bean flour. The accuracy of the method is limited only by the care with which the test is performed; that is, the ability of the operator to distinguish the most rapidly precipitating tube in a rack of ten or twelve tubes. The specificity of the method is limited only by the phylogenetic relationship of the protein mixture under test, a well-established immunological fact.

Details of the test will appear in a subsequent publication.

JOHN H. GLYNN

THE ARMOUR LABORATORIES,
CHICAGO

[1] H. R. Dean and R. A. Webb, *Jour. Path. and Bact.*, 29: 473, 1926.

[2] G. L. Taylor G. S. Adair and M. E. Adair, *Jour. Hyg. Camb.*, 32: 340, 1932.

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