A CONVENIENT, RAPID AND SENSITIVE METHOD FOR MEASURING THE INCORPORATION OF RADIOACTIVE AMINO ACIDS INTO PROTEIN

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Most of the cell-free enzyme systems currently in use for studies on the incorporation of amino acids into proteins represent relatively weak synthetic systems, i.e., incorporating only 1% or less of the added amino acid. In order to detect this low level incorporation radioactive amino acids of high specific activity (10 μc/μmole or greater) usually are added to small reaction mixtures containing limited amounts of added protein. Under these circumstances contamination of the isolated protein with radioactive nonprotein materials is great. This possibility led to the development of a highly efficient washing procedure that is now widely employed (Siekevitz, 1952). The time involved in this washing procedure, however, has severely limited the number of samples that can be processed in a given day and has imposed restrictions on the number of kinetic assays that can be carried out.

This communication describes a convenient procedure that permits the handling of 50 to 100 samples simultaneously with the same counting efficiency as the conventional method. The assay technique combines features of the filter paper assay described by Bollum (1959), the efficient washing and extraction procedure of Siekevitz (1952), and the filter paper counting procedure of Wang and Jones (1959).

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Materials and Methods. — Filter paper disks (Whatman No. 3 MM chromatography paper, 2.3 cm diameter) were numbered and mounted on straight pins to facilitate handling the disks as well as to prevent them from adhering to one another. Aliquots of the maize incorporating system (Rabson and Novelli, 1960) with C^{14} leucine as precursor were pipetted onto filter paper disks and exposed to a stream of warm air for 15 sec. The disks then were placed into an ice-cold solution which contained TCA (10% by weight) and DL-leucine-C^{12} (0.1 M). The volume of the first TCA wash was determined by the number of disks to be processed. We allowed approximately 3 ml for each disk. The acid stopped the enzymatic reaction and precipitated the protein into the matrix of the cellulose fibers. The C^{12} leucine served to dilute the radioactivity of the added C^{14} leucine. After standing for 60 min with occasional swirling, the disks were collected on a fluted filter, washed with the same volume of TCA for 15 min, and collected by filtration. Next, the disks were plunged into 5% TCA at 90° and held at this temperature for 30 min in order to hydrolyze and extract RNA and any amino acid-charged S-RNA. After collection by filtration the disks were suspended in ether-ethanol (1:1) and incubated at 37° for 30 min in order to remove TCA and lipid material. Finally the disks were suspended in ether for 15 min at room temperature for further lipid extraction and to remove water. The disks were air dried and each was placed in a standard 20 ml low-potassium glass counting vial and 5 ml of scintillation mixture was added (0.4% 2,5-diphenyloxazole, 0.01% 1,4,-di (2-(5-phenyloxazolyl)) benzene in 100% toluene). The samples were counted in a Model 314X tri-carb liquid scintillation spectrometer. Protein was determined by the method of Lowry, et al. (1951).

Results. — Fig. 1 demonstrates that, when varying amounts of radioactive protein are applied to disks and treated as described, the radioactivity observed in the protein is a linear function of the amount of radioactive protein added. Curve A represents the actual counts observed, corrected only for counter background. It is evident that the curve does not pass through the origin. When, however, the data was corrected for zero time controls, curve B was obtained. The magnitude of the zero time value is primarily a function of the

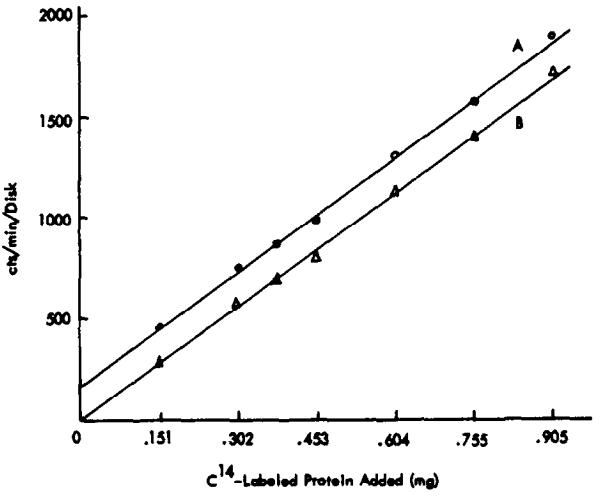

Fig. 1. C^{14} leucine was incorporated into maize particle protein for 30 min under the usual incorporating conditions (Rabson and Novelli). Increasing volumes of the reaction mixture (0.02 to 0.12 ml) were applied in duplicate to separate disks and washed and counted as described in the text. Curve A represents the average of duplicate counts corrected for background. Curve B is the same data corrected for zero time.

amount and specific activity of the added radioactive amino acid and of the efficiency of the washing procedure. In separate control experiments, not recorded here, we found that the accuracy of the counting procedure is independent of protein concentration, specific activity, or the volume of reaction mixture added, and is linear over the range tested. For this reason no complex corrections need be applied to the count obtained from each disk.

In Table 1 is presented a comparison of the new disk procedure with a modified Siekevitz method of washing the protein.

It is apparent that the disk method compares favorably with the conventional method and when the disks are counted in 100% toluene (a procedure that is not possible with soluble protein) and corrected for zero time counts the disk method is about 15% more efficient. In addition to permitting the ready processing of many samples, the disk method has the additional advantage of allowing most reactions to be run as kinetic assays rather than as one-

Table I

Comparison of the Disk Method with the Soluble Protein Method for Counting C^{14}-Labeled Protein

Time (min)	cpm/mg Protein		
	Soluble Method	70% Toluene	100% Toluene
0	209	339	523
30	2449	2062	3170
Δ	2240	1623	2647

Aliquots of a maize C^{14}-leucine incorporating system were removed at the times indicated. The data in column 2 were obtained by washing the protein by a modified Siekevitz procedure (Rabson and Novelli, 1960), dissolving the protein in 80% Formic acid, and counting appropriate aliquots in 70% toluene-30% ethanol scintillation mixture. The data in columns 3 and 4 were obtained using the disk procedure except that for column 3 the disks were counted in 70%-30% ethanol scintillation mixture and the disks of column 4 in 100% toluene scintillation mixture. The entire experiment was done in duplicate and each number represents the mean of the duplicates.

point determinations, and allowing the use of much smaller reaction mixtures, thus saving on the cost of expensive reagents and isotopes.

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