

been added containing the tracer. Usually a small amount of $N^{15}H_4^+$ is added, but any other suitable (it need not be assimilable by the agent) source of the label can be used. An aliquot of the total culture is taken for analysis in the mass spectrometer by the methods already described; then the agent is exposed to normal N_2^{14} under any conditions the experimenter wishes. After exposure another aliquot is analyzed for N^{15} , and from the decrease in atom % of N^{15} excess, fixation of the N_2^{14} is estimated.

In a detailed examination of the most suitable experimental conditions Newton¹⁵ concluded that neither accuracy nor precision was materially affected by the level of the label used; 1 to 5 atom % of N^{15} excess appeared to be most convenient. The sensitivity of the method was only about one-tenth that of a suitably designed experiment using N_2^{15} , but certain advantages may be cited. The chief one is that the test can be made under conditions best suited to the physiology of the agent and the convenience of the experimenter rather than those dictated by the closed system required for trials made with N_2^{15} . This advantage is noteworthy for experiments made with pathogenic agents with which special safety precautions must be employed. Loss of expensive N_2^{15} , particularly in long time experiments, is avoided as well as the necessity of recovering and storing the gas mixtures used. Finally, the method should be of particular value for cooperative experiments. Since the apparatus and skill required for actually running the experiment are those to be found in any laboratory, the isotopic method need not be restricted to individuals possessing both expensive physical equipment and the experience in its operation. All that the inexperienced worker need do is to arrange for a cooperating laboratory possessing a mass spectrometer to analyze the sample. This courtesy is much more readily obtained than that involving making the entire experiment with some agent of interest to only one party. An illustration of the use of the dilution method is given by Newton *et al.*¹⁶

¹⁶ J. W. Newton, P. W. Wilson, and R. H. Burris, *J. Biol. Chem.* **204**, 445 (1953).

[17] Micromethods for the Assay of Enzymes

By OLIVER H. LOWRY

In this section will be described techniques for measuring enzyme activities on a smaller scale than may be common at present (50 to 200 γ of tissue).

Several major advantages can be had from small-scale methods. There is conservation of material, either of the enzyme itself or an