

1. Radioisotope Studies in Experimental Tuberculosis

Joseph Sternberg, M. D.*

Radioisotope applications in experimental tuberculosis range from *in vitro* studies with labeled microbes to *in vivo* studies with labeled compounds in tubercular animals. The *in vitro* studies with labeled microbes are far more numerous than the *in vivo* investigations with labeled compounds or microbes.

I. Mycobacteria can be labeled in the following manners:

a. Metabolic uptake of a labeled nutrient, introduced into the culture medium; this is the case of all microbial metabolites (^{32}P , ^{35}S , ^{14}C -compounds, ^3H , etc.), or metabolic analogues (relatives), such as Selenium 75 for sulphur, Caesium 137 for potassium, Strontium 90 for calcium. Finally, in some cases, metabolites of host tissues can be incorporated by the growing microbe; this is the case of ^{14}C -labeled cholesterol or estrogens.

b. "*In vitro*" labeling of suspensions of adult cells (non-radioactive) with a radioactive element, which is selectively combined to a microbial constituent; this is the case of iodination of microbial cells, or binding of rare earths to the PO_4 free groups of nucleic acids (^{144}Ce , ^{140}La , etc.)

c. "*In vitro*" labeling of lyophilized cells, by neutron activation or by exposure to gaseous tritium (Wilzbach procedure).

Some recent experimental results will show the usefulness of radioisotope studies for the standardiza-

tion of growth conditions:

The use of highly purified glycerines for the preparation of Sauton medium is sometimes accompanied by growth inhibition and subsequent fall of the surface cellular mat. This inhibition was attributed to the removal of some trace metals indispensable for microbial growth, chiefly zinc and cobalt. In order to control the validity of this hypothesis, trace amounts of cobalt 60 were added to Sauton media prepared with glycerines of the old (growth-stimulating) type and the purified (growth-inhibiting) type. The uptake of mineral ^{60}Co was determined in both cultures and the amount of stable cobalt incorporated by the cells was calculated from the specific activity of the isotope. A significant difference was noted between microbes grown on "efficient" glycerines, and those grown on media with "inefficient" glycerines: indeed, ^{60}Co uptake in the first category was five times higher (1.46 micrograms/gram cells) than in the second category (0.5 micrograms/gram cells). The difference was noted only in young cultures (7 days), and disappeared in older cultures. Other trace metals (^{65}Zn) did not show any difference in uptake in microbes grown on either type of glycerine. Since cobalt is used by microorganisms for the biosynthesis of vitamin B_{12} , it was postulated that the minute amount of mineral cobalt added to the culture was utilized by the microbe for its own vitamin synthesis, necessary for growth. Confirmation of this hypothesis was obtained by addition of 0.5 p. p. m. vitamin B_{12} to "non-efficient" glycerines, and subsequent stimulation of microbial growth.

*Professor of Physiology and Nuclear Medicine, Faculty of Medicine, University of Montreal, Montreal, Canada.

