1. Radioisotope Studies in Experimental Tuberculosis

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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 (\$^32P\$, \$^35S\$, \$^14C\$-compounds, \$^3H\$, etc.), or metabolic analogues (relatives), such as Selenium 75 for sulphur, Caesium 137 for potassum, Strontium 90 for calcium. Finally, in some cases, metabolites of host tissues can be incorporated by the growing microbe; this is the case of \$^14C\$-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 PO₄ free groups of nucleic acids (144Ce, 140La, 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 This inhibition was attributed to the cellular mat. removal of some trace metals indispensable for microbial growth, chiefly zinc and cobalt. ocntrol 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 6°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, 60Co 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 (65Zn) 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 B₁₂, 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 B₁₂ to "non-efficient" glycerines, and subsequent stimulation of microbial growth.

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Amongst host metabolites incorporated by B.C.G., cholesterol and estrogens are being taken up by the microbe in significant amounts, when added to a modified Sauton medium. The largest fraction of the steroid remains bound to the lipidic component of the cell, but significant amounts have been detected in water-soluble extracts or in cell residues, suggesting that tubercular microbes metabolize cholesterol and estrogens, besides the well-known enzymic degradation of steroids.

II. In vivo studies with labeled microbes or microbial constituents permit a better understanding of biochemical exchanges between microbe and host. an example, a recent experiment with mycobacterial phospholipids will be presented. B. C. G. was grown in a Sauton medium with 32P; the labeled phospholipids were extracted and purified, then injected intravenously to control, tubercular and immunized guinea pig. The largest proportion of phospholipid was retained by the lungs, where it underwent a rapid catabolism; at 2 hours, the lung retention averaged 32.5% of the injected phospholipid, but the 6 hours value dropped rapidly to 2.5%. On the other hand, tubercular animals retained significantly larger amounts of phospholipids for a longer period of time (2 hours: 42.5%, 24 hours: 43.4%); animals immunized with B. C. G. showed the same values as controls.

This phenomenon was related to a change in phos-

pholipidic turnover in the lungs of tubercular guinea pig, rather than a specific metabolic pathway of mycobacterial phospholipids in tuberculosis.

III. Finally, a third example of radioisotope studies in experimental tuberculosis is that of radioiodine and labeled thyroid hormone investigations in relationship to the resistance to tuberculosis. During immunization with B. C. G. or in the early stages of tubercular infection, guinea pigs showed a significant increase in the rate of tissular utilisation of thyroid hormones, Chiefly liver, adrenals and bone marrow. On the other hand, severe tubercular infection is accompanied by a diminished utilization rate of thyroid hormones by the same tissues. This finding suggested that increased resistance to tuberculosis was accompanied by a higher utilization level of thyroid hormones, in the adrenals and the bone marrow. A confirmation of this hypothesis was made by the obtention of a significant protection of guinea pigs treated with moderate doses of tri-iodothyronine at the beginning of the infection with virulent tubercular microbes.

These examples show the versatility of radioisotope techniques in the study of experimental infection, from the research on microbial physiology to that of hormonal changes during infection. It is hoped that some of these findings will lead to practical diagnostic applications.

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