leum ether alters the permeability of the cellular surface. This can be demonstrated by testing the methylene blue reducing power of both extracted and not extracted bacilli in the Thunberg tube. It was found that under standardized conditions, all virulent strains of mycobacteria tested so far did not decolorize methylene blue, whereas avirulent strains and saprophytic mycobacteria reduced the dye. This is due to a much greater permeability of the surface of avirulent cells. After treatment with petroleum ether, virulent strains also promptly decolorize methylene blue. With respect to this test, BCG and other attenuated strains behave like virulent organisms as long as they are young, but become methylene blue decolorizing by ageing. The time required for a culture incubated under standard conditions to become methylene blue reducing is proportional to the virulence of the strain. This is in good agreement with the fact that the lipid material mentioned earlier could only be obtained from young virulent cultures and, in smaller amounts from young cultures of mycobacteria of low virulence. The possible role of this bacterial constituent is seen in its interference with phagocytes and its toxic action which manifests itself after a necessary period of sensitization in extensive hemorrhagic lesions.

*Application of Virulence Studies to the Problems of BCG Immunization**

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The immunizing effect of BCG vaccination depends upon a certain degree of multiplication of the attenuated bacilli in the vaccinated host. The extent of this *in vivo* multiplication is conditioned by a number of independent factors. The following were discussed:

a) *The receptivity of the host tissues.* Certain physiological disturbances, such as caused by malnutrition or toxic influences, may allow BCG to cause extensive lesions in experimental animals without bringing about an increase in virulence of the culture.

b) *The number of living bacilli injected.* Different batches of vaccine prepared by the standard technique and obtained from recognized BCG laboratories were found to exhibit enormous differences in their content in living bacterial cells. In all of these vaccine preparations the number of viable cells was less than 1 per cent of the total number of cells. In the absence of quantitative information concerning the viability of the bacterial suspension, it is therefore meaningless and misleading to express doses of BCG in terms of mg. or cc. A technique was described for the preparation of BCG cultures in which the numbers of living cells remained high and constant for at least six weeks.

c) *The intrinsic virulence of the BCG culture.* Four strains of BCG were found to differ greatly in several of their biological characteristics. Moreover, each of these strains proved to be heterogeneous and yielded several colonial types when cultivated on oleic albumin agar. Isolation, subculture and description of different colonial types was reported. Work is in progress to define their comparative ability to multiply *in vivo* and to induce protective immunity.

Standardization of BCG vaccination would seem to require a more quantitative control of the number of living bacteria injected and greater uniformity of the bacterial population.

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