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Morphological Elements Present in the Tubercle Bacillus Cultures.

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When tubercle bacillus cultures are ground with water and centrifuged, the largest part of the bacteria is easily sedimented, but a turbid suspension remains which cannot be cleared, even with prolonged centrifugalization. With the Sharpless super centrifuge a nearly clear fluid is obtained, and the filtration through infusoria earth or paper pulp gives a perfectly clear filtrate. After filtering the thoroughly centrifuged suspension a viscous material remains on the filter, which material can be easily distributed in water. Examination of this revealed the following characteristics: Stained according to the method of Ziehl Neelsen it takes the counter stain, very few acid-fast bacteria being present in it. More thorough examination of the preparations (the best pictures are obtained in slides not decolorized after the carbolfuchsin staining) shows that the non-acid fast material consists of bacteria and small granules. These forms, both the bacteria and the granules, even when the preparations are not decolorized, are stained neither with fuchsin nor with methylene blue, but can be seen as a negative picture between small amounts of slightly red or blue material. In the dark field numerous small granules are visible between larger forms. We obtained the best information concerning the form and size of the element in smears with argyrol. These non-colorable forms were found in large amounts in the broth cultures of 5 strains and in the culture of a freshly cultivated strain on egg medium. The described forms are present in the cultures, and are not produced through the grinding of the bacteria. This is shown when a small piece of culture is spread with gentle rubbing on a slide. The coloration of Ziehl-Neelsen and counter-staining with methylene blue reveals the presence of large numbers of them in the preparation. It is necessary to examine several areas of the slide, because in some places only acid-fast bacteria are visible, while in other places only few acid-fast forms are found in a slightly blue material. In one case, grinding about 3 gm. (dry weight) bacteria several times with water, we obtained about 0.3 gm. of this material.

The filter residue, the morphological characteristics of which have been described, has high dry material content. Its chemical examination revealed that it contains more nitrogen than the washed bac-

teria (8% and 5.6%). It contains more methyl alcohol and ether soluble material (32% and 28.5%) than the washed bacteria. After boiling 6 hours with 5% HCl we could not find reducing sugar. The washed bacteria gave about 10%. Upon the addition of acids it gives a coherent precipitate. In one case at pH 5.2 a slight, and at pH 5.0 complete precipitation occurred.

The filter residue is a very strong antigen, both *in vivo* and *in vitro*. The antigen unit in the complement fixation test was 1/30 of the antigen unit of washed bacteria. The washed bacteria cannot be well suspended in saline. The sera of guinea pigs in the course of the tuberculous infection show complement fixation more often and of higher titer with it than with any other antigen we examined. This is also the case with most of the immune sera. The material contains less of the carbohydrate precipitable substance than the washed bacteria (extraction at 100 C° with 2% Na_2CO_3 and 2% $NaC_2H_5O_2$) and somewhat more methyl alcohol soluble antigen. It gives no or only very little agglutination. The sera obtained with this material differ qualitatively from the sera obtained with the washed bacteria.

It is known that in old cultures large numbers of bacteria are dead. As tubercle bacillus cultures are always old, it is possible that the forms described in this paper are the dead bacteria. If this is the case it is remarkable that besides the loss of colorability and fragmentation, the precipitability with acids and the antigenic properties have undergone a change which does not occur in artificially killed bacteria. Certain antigenically active constituents of the cells are newly formed or become preponderant.

Do the bacteria undergo a similar change in the organism? Are the granules in the cultures alive? Do they represent the so-called filtrable forms of the tuberculous virus which plays at present a great rôle in French literature? But even without thinking of the eventual connection with important biological problems, the presence of morphological elements with changed chemical and antigenic properties is of importance for the chemical and antigenic investigation of the bacteria. The admixture of these forms, even in small proportion, to the bacillary extracts, can considerably change their antigenic properties.