Serology. — *"A Contribution to the knowledge of the nature of the Tuberculin-reaction."* By Prof. C. H. H. SPRONCK and Miss W. HAMBURGER.

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A few months ago the American researchers LONG and SEIBERT [1]) recorded that sensitized, normal tissues of tuberculous caviae do not adsorb tuberculin in vitro. They had made experiments with fine-grounded testis-tissue and skin. To 0.8 c.c. of emulsion 0.2 c.c. of tuberculin was added and the mixture was placed in an incubator for 6 hours. Subsequently it was centrifugalized and the supernatant fluid was injected into the testis of a tuberculous cavia and of a healthy one. The healthy animal showed no reaction, but a violent inflammation came forth in the tuberculous animals.

In this way, then, nothing could be observed of tuberculin combination, no more of the generation of a poisonous substance, which according to some was to arise from the tuberculin, when it is combined with its reciprocal antibody and to cause the tuberculin reaction. This induced LONG and SEIBERT to side with the adherents of SELTER's theory, which they consider, however, as "a statement of our ignorance of the true nature of the reaction rather than an explanation of it". Indeed, this somewhat singular theory runs as follows: tuberculin is a specific irritant for the sensitized cells, which poisons the cells without, however, being bound by them.

The allergy for albumins, proteins, micro-organisms, is generally attributed to antibodies, which originate in cells, especially in endothelium cells and can pass into the blood. Allowance should be made for the possibility that a substance that lacks antigenic properties, acquires them by combination with a protein. This has already been demonstrated for lipoid cell-substances.

In the investigation of the tuberculin-sensitivity of the tuberculous organism WASSERMANN and BRUCK's [2]) antigenic-antibody theory clashed with the fact that tuberculin has no antigenic properties or only very weak ones. The degree of allergy which, through repeated injection of tuberculin is difficult to elicit in healthy animals, is but the faint shadow of the sensitivity readily generated by the living bacillus tuberculosis. Nay, the question has been propounded whether the artificial tuberculin-sensitivity is, indeed, a true tuberculin sensitivity. Not without reason, since the

[1]) The Journal of the American med. Association, V. 85, N[0]. 9.

[2]) Deutsche med. Wochenschrift 1906, Nr. 12.

product called tuberculin, is a mixture of all sorts of substances, and investigators have not yet succeeded in acquiring the pure substance that is the etiological factor of the tuberculin-reaction.

Experiments on analysis of the immunity against tuberculosis [1]), which are still continued, have elucidated this problem. Filtrates of aqueous extracts of tuberculous cavia-tissue appeared to contain a substance that acts in tuberculous caviae like tuberculin, but possesses antigenic properties. In view of its active sensitization it was termed „tuberculan" (SPRONCK). Filtrates of cultures of bacilli tuberculosis contained tuberculin, never tuberculan. The reason assumed was that the bacillus tuberculosis produces in the body a complete sensitizing antigen, in vitro, however, a half, incomplete antigen without antigen power, but attracted and bound by the sensitized cells in the same way as the complete antigen. So in LANDSTEINER's nomenclature, tuberculin would be the hapten of tuberculan. In an earlier paper LANDSTEINER had already suggested the idea that tuberculan might probably be a hapten.

Moreover, we had already ascertained through our own inquiries that under the influence of repeated tuberculin stimuli the sensitized cells of the tuberculous cavia produce an antibody, that is transferred to the blood. This imparts to the serum the property to neutralize the action of tuberculin in vitro and to sensitize healthy caviae passively.

Also RUPPEL and RICKMANN's [2]) tuberculosis-serum possessed the quality to counteract tuberculin in vitro. This serum had been manufactured by sensitizing oxen and mules beforehand through infection with living virulent tubercle bacilli from man, and by obtunding after some interval of time the tuberculin-reaction by an injection of tuberculin, extracts from tubercle bacilli and living bacilli. Such a serum has latterly been applied in MOLLGAARD's sanocrysin-therapy [3]).

When tuberculin is binding with its antibody, the production of a poison in the serum is out of the question. If a poison were produced, the binding would not have been detected, as this can appear only from the harmlessness for the sensitized organism. Another explanation of the symptoms that characterize the tuberculin-reaction had, therefore, to be looked for, and we assumed with DÖRR that the physical consequences of the process of the intracellular binding produces as such the irritation, injury, necrosis of cells, inflammation, fever, and also tuberculin death.

Two years ago it was generally admitted that only living bacilli tuberculosis could sensitize the organism for tuberculin. It has since been proved that it is possible to evoke allergy for tuberculin also by injecting dead bacilli into animals and men. BESSAU [4]) and others hold the view that not the dead bacilli themselves evoke the sensitization, but the morbid growth

[1]) Ned. Tijdschr. v. Geneeskunde 1923, II Nr. 10; Medizinische Klinik 1923, Nr. 32.
[2]) Zeitschr. f. Immunitätsforschung 1910, VI, p. 344.
[3]) H. MOLLGAARD, Chemotherapy of Tuberculosis. Copenhagen, 1924.
[4]) Klin. Wochenschr. 19 Febr. 1925.

of tissue, induced by the bacilli; in other words, the tuberculous cells are assumed to produce tuberculan, whereas we think that they form anti-tuberculan. We cannot adhere to this view and will only point to the bacillus tuberculosis of CALMETTE, the so called "bacil bilié", which produces tuberculin and also sensitizes the organism, but by no means induces a morbid growth of tissue. Besides this we believe to have succeeded in obtaining tuberculan from cultures of bacillus tuberculosis also outside the organism.

It is obvious, therefore, that we felt justified in testing the above named results of LONG and SEIBERT. But their method, by which the binding of small quanta of tuberculin could not be demonstrated, has been modified by us in the following way.

We have made experiments with testis-tissue and determined in the first place the smallest quantum of tuberculin (standard tuberculin) that had to be added to an emulsion, consisting of 4 grms of testis-tissue of a healthy cavia and 6 c.c. of a physiological salt solution with which tuberculin could still be demonstrated distinctly in the filtrate (paper) of this emulsion, through intracutaneous injection of 0.1 c.c. into tuberculous caviae. It thereby appeared that the addition of 0.2 c.c. answered our purpose. It should be added that we made use of tuberculous caviae from 700 to 800 grms in weight because older animals will get more susceptible than young ones, and the susceptibility of the older animals persists so to say until death [1]). White animals were preferred, anyhow animals with a white spot on the skin of the belly, destined for intracutaneous injections after previous epilation. After these preliminary experiments we passed on to the definitive ones. A tuberculous cavia was bled to death, 4 grms of testis-tissue was weighed out and crushed in a mortar with addition of 0.2 c.c. of tuberculin, and subsequently 6 c.c. of a physiological salt solution = emulsion T (tuberculosis). In the same way we treated testis-tissue of a healthy cavia of approximately the same weight = emulsion N (normal). Both emulsions were stirred off and on, and were allowed to stand for three hours at room-temperature. Subsequently they were filtrated through paper. With these rather clear filtrates the following experiments were carried out:

1°. An intracutaneous injection was given to a tuberculous cavia, into the upper part of the belly 0.1 c.c. filtrate T, into the lower part 0.1 c.c. filtrate N.

2°. Similar injections were given to a healthy cavia.

3°. Another healthy cavia received under the skin of the upper part of the belly 2 c.c. filtrate T and in the lower part 2 c.c. filtrate N.

As to distinctness the result of these experiments left nothing to be desired. The healthy caviae evinced not the slightest of reaction. In the case of the tuberculous cavia, on the contrary, a distinct tuberculin-reaction (a small cockade with infiltration and a trace of necrosis in the center)

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[1]) R. DEBRÉ et H. BONNET. C.R. de la Société de Biologie, T. 86, p. 485.

manifested itself regularly on the site of inoculation of filtrate N. This reaction persisted for many days. In the upper part of the belly, at the site of inoculation of filtrate T either some red colouring appeared, that faded already after a couple of days, or no reaction followed at all.

It cannot be mistaken, therefore, that in vitro normal, sensitized testis-tissue of tuberculous caviae adsorbs tuberculin. But of the hypothetical poison, that was supposed by some to be produced, we could no more than LONG and SEIBERT detect anything. We have not tried to determine how much tuberculin is adsorbed by sensitized testis-tissue, because this quantity may be considered to depend upon the degree of sensitization. But 1 grm of testis-tissue adsorbed, as we think, certainly not more than 50 mgr. of tuberculin, so that the result of the experiments by LONG and SEIBERT is quite intelligible, as they had added more than 200 mgr. per gram of tissue.

The adsorption of tuberculin by sensitized tissue, which is readily demonstrable by our method, is quite in harmony with the antigen-antibody theory and goes against SELTER's theory. Our simple method, which may still be improved, can also be applied to compare the sensitivity of different organs of a tuberculous animal.

A short time ago DIENES and FREUND [1]) reported inter alia that they could not demonstrate tuberculin in the urine after the injection of an aqueous extract of bacilli tuberculosis into tuberculous caviae apparently on account of binding with sensitized cells ; in healthy caviae on the other hand, which had received an equal dosis of the same extract, they found a large quantum of tuberculin in the urine. Clinical experience also lent support to the antigen-antibody theory. In their recently published "Leerboek der Longtuberculose" VOS and LEUSDEN state : „In practice we can attain to the best and persistent results with the treatment of those patients with whom desensitization can be obtained to high doses of tuberculin" [2]). As regards tuberculin-treatment the tuberculosis-specialists are divided into two groups. The allergists strive for the maintenance of tuberculin sensitivity ; the anergists on the contrary try to obtund it. The allergists receive support from SELTER's theory, the anergists from WASSERMANN and BRUCK's antigen-antibody theory, as modified by us. According to the latter theory the favourable action of the obtusion of the sensitivity, is easy to understand. Allergy of the tuberculous patient indicates a lack of antibodies in the blood, and that, owing to this the cells are not protected against tuberculan, which is transferred spontaneously from the tuberculous foci to the blood. Through an appropriate tuberculin treatment the sensitized cells are incited to secrete antibodies, which circulate in the blood and cause a decrease of the tuberculin-allergy, because the tuberculin that

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[1]) DIENES and FREUND, American Review of Tuberculosis, Baltimore, Bd. 12, Sept. 1925.

[2]) B. H. VOS and J. TH. LEUSDEN, Leerboek der Longtuberculose. Utrecht. A. OOSTHOEK, 1925, Dl. II, p. 233.

is introduced, has already been saturated with antibodies in the blood, and consequently does not reach the cells. The obtunding treatment transfers the process of the binding which is deleterious to the cells, from the cells to the blood, where the binding is not noxious. So the obtusion promotes the rest of the sufferer, because it promotes the relaxation required for the healing of the tuberculous foci, since the foci can no more stimulate and deteriorate each other through spontaneous inoculation of tuberculan into the blood. Thus the generally admitted favourable effect of the rest-cure is accomplished by obtusion of the tuberculin-allergy.

From the Bacteriotherapeutic Laboratory at Zeist.)