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Identification of Skin-reacting Substance in Blood Sera from Tuberculous Patients and Animals.

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A biologic method has been devised for identifying a specific skin-reacting substance in the blood serum of tuberculous patients and animals. The point of departure was an earlier observation reported elsewhere[1] that sensitization in normal guinea pigs could be accomplished with fractional tuberculins prepared from non-protein substrates.

Duplicate series of healthy white guinea pigs, weighing 450 to 500 grams, were used in each set of experiments. These were grouped as follows:

(1) Normal guinea pigs previously injected with tuberculin fractions.

(2) Normal, untreated guinea pigs, and

(3) Tuberculous guinea pigs with early, moderately advanced, and far advanced infections.

The animals were tested with (a) human sera from early and far advanced tuberculosis, (b) same sera heated at 60 to 65° C. on a water-bath for 45 minutes, (c) normal human serum, (d) guinea pig serum from early and moribund stages of tuberculosis. Intracutaneous injections of 0.02 cc. of each serum were given for each test. The guinea pigs of the first series were injected subcutaneously with increasing doses of one of the fractional tuberculins over a period of 7 weeks, receiving a total amount of 2.2 cc. of each substance. Ten to 14 days after the last injection, intracutaneous tests were applied with the materials described. Reactions were recorded after 24, 48, and 72 hours, and observations made until the sites of injection appeared normal.

Groups of normal, sensitized, and tuberculous guinea pigs react differently toward intracutaneous injections of minute amounts of blood serum from tuberculous patients or animals. The specific skin reactions appear to depend upon the stage of infection to which the serum corresponds. Observed differences are

referable to an early or far advanced tuberculous process, and if this has been arrested, to the presence or absence of toxemia. This relationship of serum and its source is influenced, moreover, by the stage of tuberculosis in the test animal. Furthermore, heating tuberculous serum at a temperature of 60 to 65° C. reduces a skin reaction markedly or extinguishes it completely when all other conditions are kept constant. Normal guinea pigs, for example, react positively toward tuberculous serum but do not give a reaction with the same serum heated. Similar effects are noted in the group of animals sensitized by repeated injections of tuberculin fractions, and also in the groups of tuberculous animals. Sera from tuberculous guinea pigs in a moribund state give a delayed and transitory skin reaction or a negative one in animals ordinarily responding in varying degree to sera from early or advanced tuberculosis. Finally, normal human or guinea pig serum fails to elicit any reaction in the different groups of animals. As evidence of a more general tissue response in reacting guinea pigs, there is a pronounced inguinal adenopathy.

The mechanism of the reactions can be explained on the basis of addition or subtraction of one or more interacting substances in tuberculous sera and in the tissues of the experimental animal. For example, a group of normal tuberculin-sensitized guinea pigs reacts more violently and persistently to intracutaneous injections of serum from advanced toxemic tuberculosis than to serum from an early, non-toxic, arrested infection. This group of animals is strongly endowed with tuberculin-reacting elements and with antibodies for products of tubercle bacilli. Injection of the serum causes an augmented reaction by introducing appreciable amounts of toxin. In the same group, however, the results are negative when heated serum is used, indicating that toxin is not introduced. In the untreated normal guinea pigs, the same holds true because of the interaction of toxin and normal antibodies.

A heat-sensitive skin-reacting substance of the nature of a toxin has been identified in the blood serum of tuberculous patients and guinea pigs. It is not found in normal human or guinea pig serum. The substance does not behave like tuberculin or its related elements which are heat-resistant, and exists independently of these. It is present in largest amounts in far ad-

vanced tuberculous infections with profound toxemia, and is apparently destroyed by heating at 60 to 65° C. for 20 to 45 minutes. In normal animals this unheated substance gives a positive skin test owing to its interaction with normal antibodies for tubercle products.

The method devised for identifying this specific skin-reacting substance depends, in principle, upon a living "indicator", the guinea pig, which measures related or identical elements in the serum to be tested. The procedure was made possible by a previous demonstration that normal animals could be sensitized with fractional tuberculins prepared from non-protein substrates.

Skin reactions obtained according to the technic described in these experiments are referable to an addition or subtraction of one or more of the interacting substances present in tuberculous serum and in the tissues of the experimental animal.

Evidence is adduced to show that the heat-labile substance, probably a toxin, in tuberculous sera, can be used as a measure of the circulating antibodies in normal and tuberculous persons. A test of this type may serve as an index of the bodily resistance to tuberculous infection in both groups.

Certain theories and hypotheses regarding tuberculin and skin sensitiveness find controllable experimental evidence in the observations described. An explanation is offered for negative tuberculin tests in far advanced tuberculosis, and furthermore, positive reactions with tuberculous sera in apparently normal persons are to be attributed to a toxin. Accordingly, the results with the autoserum test of Lenz[2] and modified autoserum[3] and Wildbolz reactions[4] take on a somewhat different interpretation.

Studies are now in progress to determine the value of a test which is fundamentally like the Schick test in diphtheria, and the Dick test for scarlet fever. Already experimental evidence is at hand indicating the presence of a toxic substance in filtrates obtained from the growth of tubercle bacilli in special culture media.

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[1] Eberson, F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 508; *Am. Rev. Tuberc.*, 1926, xiii, 454.

[2] Lenz, W., *Schweiz. med. Wchnschr.*, 1920, l, 321.

[3] Henrichsen, K. J., and Sweany, H. C., *Am. Rev. Tuberc.*, 1923-1924, viii, 341; *ibid.*, 1923-1924, viii, 359.

[4] Enright, J. J., and Rettger, L. F., *Am. Rev. Tuberc.*, 1924, x, 104; Von Bergen, J. O., *Schweiz. med Wchnschr.*, 1921, xli, 655; *ibid.*, 1921, lxviii, 733.