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On the specificity of an anti-leprosy serum.*

by

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The method of preparation of the anti-leprosy serum concerned has been described in detail in some previous papers by the author (1, 2, 3). Briefly, it consists of repeated injections into sheep of increasing doses of toluol-treated, fresh and older glycerin bouillon cultures of Kedrowskij's Russian strain (isolated from a leproma) and Reenstierna's Swedish strain (isolated from the blood of a leper), which contain both acid-fast and non-acid-fast life-forms of the mycotic micro-organism of leprosy, together with their desintegration products and toxins. Each animal is given equal quantities of the two antigens, the former denoted M (=Moscow), the latter R. Thus, the serum used for therapeutic purposes will be an M+R-serum. For certain laboratory researches, moreover, an M-serum and an R-serum were prepared according to the aforesaid method, i. e., one sheep was given injections of M-antigen only, another of R-antigen.

Thanks to the kindness of Professors T. Svedberg and A. Tiselius and some of their assistants, especially Dr. H. Svensson, *electrophoretic analyses,* with the use of the Tiselius apparatus (4), have been carried out at the Institute of Physical Chemistry in Upsala on the following sera from sheep, all preserved with 0.5 per cent phenol: one each M- and R-serum, two M+R-sera (Nos. 35 and 36) which had been tried with good therapeutic results on leper patients in different countries (5), and two normal sera. The results of the analyses are rendered in the ensuing diagrams and table.

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Diagrams

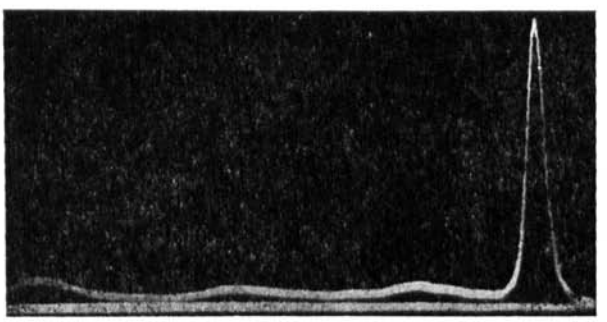

Fig. 1

Normal sheep serum No. 1. Exposure taken in the positive limb after 330 minutes' migration. Potential gradient 4.9 volts/cm.

The diagrams 1-4 are reproductions of four exposures made during the electrophoretic experiments with the Philpot-Svensson optical arrangement (6, 7). This method gives on the photographic plate a curve, whose ordinate is proportional to the concentration gradient, and whose abscissa is proportional to the displacement of the gradient in the U-tube. Each peak in the diagram corresponds to a more or less well-defined component of the serum, and the area between this undulating curve and the base-line is a measure of the concentration of that component. A mechanical integration of such a diagram renders thus a quantitative analysis of the serum. The results of these integrations have been summarized in the table. It should be noted, however, that the figures in the table are averages of several exposures of both U-tube limbs.

The diagrams 1 and 3 show exposures of the two normal sheep sera (Nos. 1 and 2), from the positive and negative limb, respective-

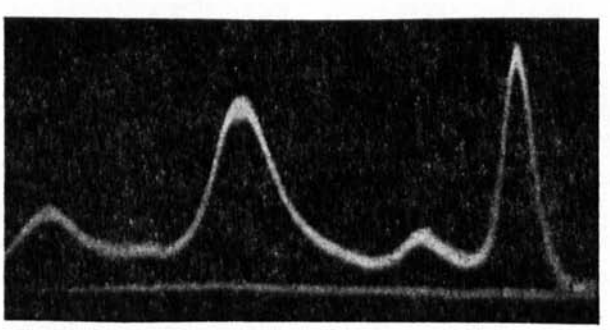

Fig. 2

Anti-leprosy serum M. Exposure taken in the positive limb after 325 minutes' migration. Potential gradient 4.8 volts/cm.

ly. The direction of migration is from left to right in diagram 1, and oppositely in diagram 3. The large peak represents serum albumin, the fastest component of the serum. Then follow the globulin components, α, β, and γ. The last peak of each diagram represents no real component, but is an anomaly that always occurs in electrophoretic experiments with a fairly high protein concentration (8).

The diagrams 2 and 4 show exposures of two numbers (M and 36) of the said anti-leprosy serum. Diagram 2 is of the positive limb, thus directly comparable with No. 1, and 4 of the negative limb, comparable with No. 3. The great relative increase in the γ globulin fraction and the decrease in the albumin fraction, are very striking.

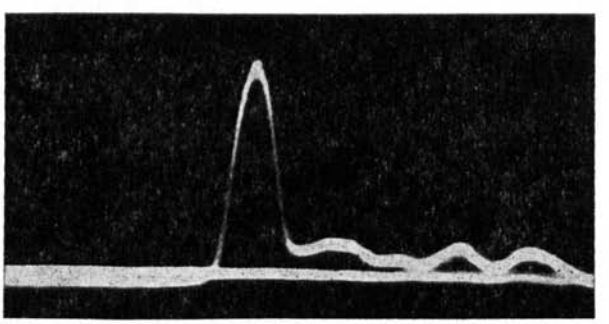

Fig. 3

Normal sheep serum No. 2. Exposure taken in the negative limb after 202 minutes' migration. Potential gradient 5.6 volts/cm.

The difference in electrophoretic composition — demonstrated by the diagrams and the table—between the anti-leprosy and the normal sera is greater than that between all other immune sera (anti-diphtheritic, anti-pneumococcic, etc.) and corresponding normal ones which have previously been analyzed at the Institute of Physical Chemistry. Since antibodies are now known to belong to the γ fraction, or at least to a slowly moving globulin, of sera, their very considerably increased percentage in the anti-leprosy serum in question must be considered as noteworthy.

The immune sera M, R, 35, and 36 had previously been tested at the Institute of Hygiene and Bacteriology in Upsala by Dr. V. Hallberg in regard to their possible capability of *complement fixation* by the use, as antigen, of methanol extracts (according to Bouquet and Nègre) of the strains M and R, as well as of an acid-fast strain isolated by Lleras Acosta from the blood of a Colombian leper. The tests of the four immune sera then resulted in complete deviation of the complement regarding all three antigens, while two

Serum fractions in per cent of total protein.

	Albumin	Globulin		
		α	β	γ
Normal serum No. 1.....	68.5	14.7	3.0	13.8
" " " 2.....	65.35	16.2	6.0	12.45
Anti-leprosy serum M.....	30.8	12.5	9.2	47.5
" " R.....	29.8	15.9	15.0	39.3
" " No. 35....	27.6	15.9	17.4	39.1
" " " 36....	29.7	16.2	17.0	37.1

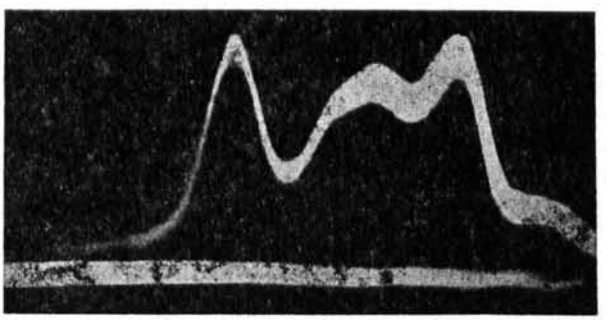

Fig. 4

Anti-leprosy serum No. 36. Exposure taken in the negative limb after 235 minutes' migration. Potential gradient 4.8 volts/cm.

TABLE

normal sheep sera did not prevent the appearance of complete lysis. In this connection should, moreover, be mentioned the interesting fact that sera from two Javanese lepers (nodular form) also gave a strongly positive reaction when the R- and the Lleras Acosta-antigen were used, and a partial one in the case of the M-antigen. On the other hand, a serum from a Javanese patient suffering from leprosy of the neural type showed a negative reaction to the three antigens[1] in the same way as a normal human serum.

The laboratory experiments mentioned in the preceding go to show that serum from sheep immunized with two bacterial strains isolated from lepers differs very considerably from corresponding normal serum in those respects which one is accustomed to asso-

[1] The Javanese sera provided by Drs. J. B. Sitanala and R. M. Djoehana Wiradikarta, had prior to their dispatch to Upsala been tested at the Bacteriological Laboratory of the Government Hospital in Semarang by Dr. M. Sardjito, who used as antigen an alcohol-ether extract of a strain isolated by him from a nodule of a lepromatous Javanese case. By this means he was able to establish that the two sera from nodular leprosy behaved strongly positive, and that from neural negative.

ciate with the presence of antibodies. This fact strengthens the author's previously advanced opinion that the therapeutic results obtained with the anti-leprosy serum in question are partially due to a specific action.

However, a precise knowledge of the therapeutic specificity of an immune serum can merely be gained through comparative tests with corresponding normal serum, carried out in man on a very large scale[2] and on fairly equivalent material. But the carrying out of such large series with normal serum, permissible in animals, will meet such great obstacles that they have never been made in human beings.

Therefore, nobody has as yet succeeded in determining how much of the therapeutic action of e. g. anti-diphtheritic serum is really specific. The same holds good for chaulmoogra oil and its derivatives. The specificity claimed for them when acting on leprous lesions has in no way been proved. Nevertheless, the use of anti-diphtheritic serum has been going on for more than 50 years and that of chaulmoogric preparations for centuries.

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[2] To a previous report (1, 2) on control tests with normal sheep serum in lepers, on a smaller scale, resulting negatively should in this connection be added a recent one, i. e., a personal communication (Bogota in August, 1943) from the Chief of the Department of the Anti-Leprosy Campaign in Columbia, Dr. M. Bernal Londoño, stating that normal sheep serum used there in leprosy treatment has proved ineffective.