

### Antibody Heterogeneity to *Trypanosoma cruzi*

It is well established that antibodies in mammals belong to three related classes of serum proteins: IgG, IgM, and IgA<sup>1</sup>.

There are, however, some data attributing antibody activity to other serum fractions. In tuberculosis, under certain circumstances, increases of the  $\gamma$ - or  $\alpha$ -globulins demonstrated by electrophoresis were associated with the presence of antibodies in rabbits<sup>2</sup> and in humans<sup>3</sup>. Using radio-immunoelectrophoresis, antibodies to *p*-azobenzene-arsenate were found in a  $\beta$ -1 globulin fraction in rabbits immunized with this hapten, conjugated to bovine  $\gamma$ -globulin<sup>4</sup>.

Sera from rabbits immunized with a strain of insect flagellates were shown to have  $\alpha$ -1 globulin mobility<sup>5</sup>.

In the present experiments, antibodies were produced in rabbits against a strain of *T. cruzi*, non-pathogenic for mice. The preparation of the antigen and the immunization scheme were carried out as previously described<sup>6</sup>.

Immunoelectrophoretic analyses of the antisera developed against a *T. cruzi* ultrasonic extract (5 mg protein/ml) showed, in addition to a precipitin arc in the  $\gamma$ -globulin zone, another line extending in the  $\alpha$ -2 globulin area (Figure 1). After chromatography on DEAE cellulose, a fraction eluted with 0.0175 *M* phosphate buffer at pH 6.3 showed only the IgG globulin arc when tested by immunoelectrophoresis against the homologous antigen.

The same anti *T. cruzi* serum was precipitated with rivanol<sup>6</sup> and the fraction obtained was tested by immuno-

electrophoresis against a *T. cruzi* ultrasonic extract and against a guinea-pig anti-rabbit serum lacking anti- $\gamma$ -globulin antibodies (Figure 2). Two precipitin arcs developed against the *T. cruzi* antigen; one with the mobility characteristic for IgG, the other completely separated and displaying an unusually rapid rate of electrophoretic migration. The position of the precipitin arc formed between this rivanol purified fraction and the guinea-pig anti-rabbit serum shows that this fast-moving precipitin arc might have an  $\alpha$ -2 globulin mobility. Electrophoresis on cellulose acetate showed that this serum fraction contained mainly  $\gamma$ - and  $\alpha$ -2 globulins (Figure 3).

Since non-specific reactions are known to occur between certain macromolecules and serum proteins, normal sera and serum fractions prepared in the same way were tested against *T. cruzi* antigen by immunoelectrophoresis. The tests were negative throughout.

The present observations show that the precipitating activity present in an electrophoretically fast-moving serum fraction corresponds to a specific antibody. They also show that antibodies to *T. cruzi* produced in rabbits may display an outstanding electrophoretic heterogeneity. In a previous paper<sup>6</sup>, another strain of hemoflagellates was shown to determine the production of electrophoretically fast-moving antibodies. The production of such antibodies therefore seems to take place in response to antigens belonging to a group of biologically related organisms. The known high lipid or lipoprotein content of the hemoflagellates might have some bearing on this phenomenon. The use of immune-specific methods would eventually permit the isolation of the antigenic determinants involved.

A thorough characterization of this unusual antibody would show whether or not it shares common structural features with the three well-described classes of immune globulins.

An antibody with completely distinctive physico-chemical and antigenic characteristics would have important implications regarding the mechanisms of the immune response and its cellular origin<sup>7</sup>.

**Résumé.** Des lapins immunisés par des cultures de *Trypanosoma cruzi*, ont produit des anticorps qui, examinés par analyse immunoélectrophorétique envers un extrait antigénique homologue, ont démontré une hétérogénéité électrophorétique exceptionnelle.

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Fig. 1. Immunoelectrophoretic pattern of anti *T. cruzi* serum. Stained with nigrosin.



Fig. 2. Immunoelectrophoretic pattern of rivanol purified anti *T. cruzi* serum. Upper channel: *T. cruzi* ultrasonic extract. Lower channel: guinea-pig anti-rabbit serum. Stained with nigrosin.

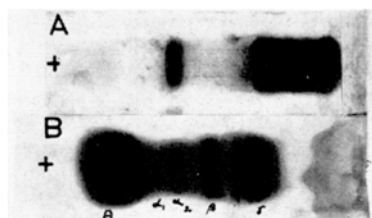


Fig. 3. Cellulose acetate electropherograms. A, Rivanol purified fraction. B, Whole anti *T. cruzi* serum. Stained with nigrosin.

<sup>1</sup> Nomenclature for Human Immunoglobulins. W.H.O. Bull. 30, 447 (1964).

<sup>2</sup> F. SEIBERT, Perspect. Biol. Med. 3, 264 (1963).

<sup>3</sup> J. ALLERHAND and C. M. ZITRIN, J. Immunol. 89, 252 (1962).

<sup>4</sup> K. ONOUE, Y. YAGI, and D. PRESSMAN, J. Immunol. 92, 173 (1964).

<sup>5</sup> G. STREJAN and I. FLECHNER, Proc. Soc. exp. Biol. Med. 115, 352 (1964).

<sup>6</sup> J. HORESI and R. SMETANA, Acta med. scand. 155, 65 (1956).

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