ISOLATION OF THE CENTER OF SPECIFIC ACTIVITY
OF AN ANTIBODY FROM AN ANTISERUM HYDROLYZED
WITH PAPAIN, USING AN ANTIGEN FIXED ON CELLULOSE

A. Ya. Kul'berg and I. A. Tarkhanova

Division of Biochemistry of the N. F. Gamaleya Institute of Epidemiology
and Microbiology, Moscow

(Presented by Active Member AMN SSSR L. A. Zil'ber)

(Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 76-79, November, 1960

Original article submitted October 10, 1959

It has previously been shown [4-7] that, as a result of the action of the proteolytic enzyme papain on an
immune globulin, the molecule of the latter is split up into several parts, without loss of the power of this γ-
globulin to react specifically with its own antigen.

All attempts to isolate from the products of proteolysis in a pure form the fragment of the globulin mole-
cule possessing the specific antideterminant, using chemical or physicochemical methods [6, 7] for this purpose, have, however, been unsuccessful.

It accordingly appeared to be of great interest to use the specific antigen-antibody reaction to isolate the
active center of the antibody from the products of proteolysis, using for this purpose an antigen fixed on an in-
soluble carrier (cellulose).

EXPERIMENTAL METHOD AND RESULTS

The immune sorbent in our experiments was horse albumin, fixed on cellulose by Campbell's method [3],
which we modified.*

A rabbit immune serum against crystalline horse albumin was fermented with papain** for 16 hours at 37°.
The papain was preliminarily activated by being allowed to stand in a buffer solution at pH 7.0, consisting of
equal parts of a 0.05M solution of cystein hydrochloride, a 0.01M solution of Na-verseue and a 0.3M solution of
K₂HPO₄. The duration of activation was 1 hour at 42°. The quantity of papain added to the serum was 0.5 mg
for each 70 mg of serum protein.

After the completion of the fermentation of the serum, it was cooled to 2°, to it was added a 0.05M solu-
tion of monolodoacetate (papain inhibitor), and it was then subjected to rapid dialysis in the cold against physio-
logical saline. Dialysis continued for 28-30 hours.

The experiments showed that a serum, when fermented in this way, completely lost its precipitating pro-
perties, but at the same time preserved its power of specific combination with its antigen (horse albumin), which
could be judged by the ability of this serum to specifically inhibit the precipitation reaction between horse
albumin and unchanged serum. In subsequent work this test has been used for detection of the specific anti-
determinant in a fermented serum.

* In contrast to Campbell, the cellular tissue was not combined with p-nitrobenzylchloride but with p-nitro-
benzylbromide, which has a much higher reactive power.

** The preparation of papain was obtained from the Baker Research Laboratory, London.
Protein Content of Specific Precipitate (in mg) Obtained as a Result of the Reaction between a Fermented Rabbit Immune Serum (Antigen) and an Ass’s Serum Against Rabbit Globulin

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Quantity of antigen (in mg)</th>
<th>Quantity of protein in specific precipitate (in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.005</td>
<td>0.05</td>
</tr>
<tr>
<td>Fermented serum</td>
<td>0.223</td>
<td>0.344</td>
</tr>
<tr>
<td>Fermented serum, exhausted with Immune sorbent</td>
<td>0.172</td>
<td>0.297</td>
</tr>
<tr>
<td>Eluate from Immune sorbent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. In the experiments 0.25 ml of antiserum, diluted 1:2, and a corresponding amount of antigen were used.

The presence of the antigenic determinant of the \( \gamma \)-globulins (b) and the antideterminant of the antibody (a) in an immune serum after fermentation with papain, in the same serum when exhausted with immune sorbent, and in the eluate from the immune sorbent.

In order to isolate the specifically active center of the antibodies, the immune serum, after fermentation as described above, was mixed with the immune sorbent for 2 hours at room temperature, after which the supernatant fluid was tested for its content of antideterminant in inhibition experiments. These experiments showed that the immune sorbent completely extracted from the serum the fragment of the \( \gamma \)-globulin molecule possessing specific antibody activity, as a result of which the serum, after exhaustion by the immune sorbent, lost its ability to specifically inhibit the precipitation reaction.

After the thorough removal of proteins not reacting with the immune sorbent, by means of the repeated washing with physiological saline on a Büchner funnel, elution of the specific inhibiting factor from the immune sorbent was carried out. This elution was done by acidification to pH 2.6 for 2 hours at room temperature, after which the presence of specifically active centers of the antibody was determined in the eluate.

The experiments showed that the eluate does, in fact, contain the antideterminants of the antibodies, as disclosed by its ability to specifically inhibit the precipitation reaction between the unchanged serum and its antigen.

Determination of the protein content in the eluate showed that, taking account of all the volume changes, it comprised one third of the total protein content of the antibodies in the test serum (8.6 mg antibody protein