ANTICOMPLEMENTARY FACTOR IN FRESH YEAST*

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Although the inactivation of complement by yeast cells was described by von Dungern (1) as early as 1900, the nature of the phenomenon has remained obscure.

It has been established by Coca (2) that the inactivation by yeast is due to the removal or destruction of a relatively heat-resistant fraction of complement, and not to the introduction of inhibiting substances. Furthermore, Whitehead, Gordon, and Wormall (3) employed a zymin preparation, and found that it combined either physically or chemically with the third component of complement.

The present report deals with the preparation of an insoluble fraction from fresh yeast which can inactivate, specifically, the third component of complement.

Materials and Methods

The method of McAnally and Smedley-MacLean (4) was used to fractionate the yeast, but the employment of 40 per cent KOH, as called for in this method, appeared to be too drastic, inasmuch as the resultant insoluble fraction had little or no ability to inactivate the third component of complement. The method employed for the preparation of the insoluble, active fraction follows.

500 gm. of fresh Fleischmann’s yeast were suspended in 2 liters of 0.5 M Na₂HPO₄, boiled for 1 hour, and then cooled. 0.5 gm. of purified trypsin (Wilson special trypsin) was added together with a few drops of toluene, and the mixture was incubated at 37° for 16 days. At the end of this time the mixture was negative.

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to protein tests. The supernatant fluid was decanted and discarded. 2 volumes of 95 per cent ethyl alcohol were added to the residue; the latter was then filtered and washed six times with 95 per cent alcohol. The supernatants and washings were discarded. The precipitate (Fraction A) was added to 4 liters of warm water, boiled for 30 minutes, and allowed to settle; the supernatant was syphoned off. This process was repeated two times. The original precipitate (Fraction A) was boiled several times with distilled water and centrifuged each time while hot, the process being repeated until the acid hydrolysis products of the supernatant liquids failed to reduce Fehling's solution. The residue was washed repeatedly with absolute alcohol and dried as completely as possible. The dried material was then refluxed for 3 hours with absolute alcohol, filtered, and finally dried in vacuo. The resultant product was a fine, almost white, hygroscopic powder, which was insoluble in hot water, organic solvents, and cold alkali. The yield was about 2 per cent. Chemical analysis revealed carbohydrate 94 per cent, nitrogen 1.78, magnesium 2.43, phosphorus 0.4. This fraction was labeled the "insoluble fraction," and it proved to be the agent responsible for the inactivation of the third component.

This material was further fractionated by the following procedure.

2 gm. of the insoluble fraction were added to 100 cc. of cold 1 N HCl, and the mixture was kept overnight in the refrigerator at a temperature of about 3°. The suspension was then centrifuged, and the supernatant, which contained the magnesium-phosphorus complex (4) was decanted. The precipitate (Fraction B) was added to 1 liter of warm water, boiled for 1 hour, and centrifuged while hot. The undissolved material was boiled again several times with distilled water, and processed by a method similar to that used in the preparation of the original insoluble fraction. The product was designated as Fraction C, and contained 0.54 per cent nitrogen. All of the insoluble fractions were stored in vacuo, since, upon long exposure to air, they adsorbed moisture and changes occurred so that they no longer inactivated the third component. When such changes occurred, the original activity was restored by refluxing the material again with absolute alcohol.
Immunological

The methods employed in this laboratory for complement titrations and for reactivation procedures have been previously described (5). The following method was used in testing the fractions.

Various amounts of the insoluble fractions were added to 50 volumes of distilled water, boiled for 30 minutes, and centrifuged. The supernatants were drained, and the residues allowed to cool. 1 cc. of fresh guinea pig serum was added to each fraction, thoroughly mixed with the fraction, and the suspensions were placed in a water bath at 37°. The mixtures were shaken by hand every 10 minutes. After incubation for 2 hours, the mixtures were made up to 10 cc. with 0.9 per cent NaCl solution and centrifuged clear. The pH of the supernatants was adjusted to 7.2, and complementary activities were immediately determined. Reactivation experiments were carried out directly afterward. The soluble fractions were added directly to 1 cc. of serum, and treated thereafter in the same manner as were the insoluble fractions. The amounts employed and the anticomplementary action of each fraction are given in Table I.

Table I
Action of Various Constituents of Yeast on Third Component of Complement

<table>
<thead>
<tr>
<th>Yeast fraction</th>
<th>Minimal amount necessary for inactivation of third component of complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction A</td>
<td>250</td>
</tr>
<tr>
<td>“ B</td>
<td>50</td>
</tr>
<tr>
<td>“ C</td>
<td>50</td>
</tr>
<tr>
<td>Insoluble fraction</td>
<td>10</td>
</tr>
<tr>
<td>Fresh yeast</td>
<td>250</td>
</tr>
</tbody>
</table>

Results

It is seen in Table I that the insoluble fraction inactivated the third component of complement in amounts only one-twenty-fifth of the required amount of fresh yeast. After the cold HCl treatment of the insoluble fraction, which resulted in a loss of 25 per
cent of the carbohydrate, a loss of 70 per cent of the total nitrogen, and a loss of 75 per cent of the magnesium-phosphorus complex, the insoluble fraction still possessed marked anticomplementary power. *None of the soluble fractions inactivated the third component.* Since all of the active, isolated fractions were insoluble, it is altogether possible that the inactivation of the third component is due to an adsorption of a heat-resistant component of blood serum. Further study of the phenomenon is in progress.

**SUMMARY**

An insoluble fraction isolated from fresh Fleischmann's yeast is the agent responsible for the anticomplementary properties of the yeast.

It is suggested that the inactivation of the third component of complement is due to the adsorption of a relatively heat-resistant fraction of blood serum.

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