TABLE II. Antimony Content of Samples from Blood of Rat.

Sample	c/min./g
Defibrinated blood	8600
Plasma	65
Hemoglobin (aluminum hydroxide method)	73800
Hemoglobin (centrifugation only)	74100

have been loosely attached to the hemin, but it seems more likely that it was attached to the protein portion of the hemoglobin. An added reason for believing this is the fact that the red cells of the dog do not seem to take up the antimony nearly so avidly as those of the rat. Presumably there is no difference between the hemin of the rat and of the dog, but the protein portions of the molecules are different. Support by analogy is found in the fact that arsenic, a member of the same family of elements as antimony, when administered as sodium arsenite, has been shown to be taken up by the red cells of the rat and to go with the globin and acid-acetone soluble heme fractions(5).

Summary. Labeled fuadin prepared from neutron irradiated antimony trioxide has been used in a study of the localization of antimony in the red cell fraction of rat blood. The results are consistent with the hypothesis that the antimony is attached to the globin portion of hemoglobin.

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The Polysaccharides of *Candida albicans.** (21103)

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The presence of serologically active polysaccharides in organisms belonging to the genus Candida has held the attention of many investigators interested in devising serologic tests for the diagnosis of Moniliasis, and demonstrating cross reactions between Candida and other closely related yeast-like organisms (1-3). The present report studies the homogeneity of a *Candida albicans* polysaccharide preparation by quantitative immunologic technics.

Materials and methods. A strain of *Candida albicans*‡ giving typical chlamydospore formation on corn meal agar, sugar fermentation reactions consistent with the species, and requisite pathogenicity in the rabbit was selected for use in this work. The organism was cultured on Sabouraud's agar in Roux flasks at 30°C for 3 days. Growth was washed off gently with acetate buffer, pH 6.03, and adjusted to approximately 50% concentration by volume. The suspension was autoclaved at 15 lb pressure for 10 minutes and then centrifuged to recover the supernatant. Addition of one volume of 95% alcohol precipitated the polysaccharide and protein constituents, which after solution in distilled

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water were treated by Sevag's method to free it from protein. The purified polysaccharide, after additional alcohol precipitations in the presence of 10% sodium acetate and 1% acetic acid, was finally dialyzed against distilled water and dried by lyophilization. All procedures were carried out at 0-4°C. Gray rabbits weighing approximately 2 kg were injected intravenously with a heavy suspension of heat-killed *Candida albicans* cells over a 3-week period. Animals were injected 3 consecutive days of each week with increasing amounts of antigen. High titered serum was obtained by bleeding the rabbits 6 days after the last injection. The quantitative test was used and the technics outlined in Kabat and Mayer(4) for treating antigen-antibody precipitates prior to nitrogen determinations were followed. One ml of a 1:1 dilution of antiserum was added to tubes containing 4 ml of antigen dilution prepared in saline. The total antibody nitrogen was estimated quantitatively by a modification of the biuret method suggested by Robinson and Hogden(5). A standard curve was drawn from spectrophotometric density readings at 540 mM of the biuret color developed from amounts of purified egg albumen ranging from 0.5 to 10 mg of protein. The biuret color developed by antigen-antibody precipitates was read on this curve and the antibody nitrogen estimated by multiplying the protein value by 0.16.

Results. The values for antibody nitrogen precipitated by added antigen appears consistent with a single antigen-antibody system throughout the range of antibody excess; however, the absence of a clear-cut equivalence zone as determined by supernate analysis, and the persistence of insoluble antigen-antibody complexes with a marked increase in total antibody precipitated in the region of antigen excess, strongly suggests the inhomogeneity of the polysaccharide preparation(6) (Fig. 1 Curve A). Attempts were made to fractionate the polysaccharide preparation into 2 components by precipitation with saturated lead acetate. On one occasion lead precipitation removed a yellow, gummy material, apparently nucleic acid, since U.V. absorption at 2600 Å of material remaining in the supernate was greatly reduced after treatment. Separation of the 2 components, however, was not successful.

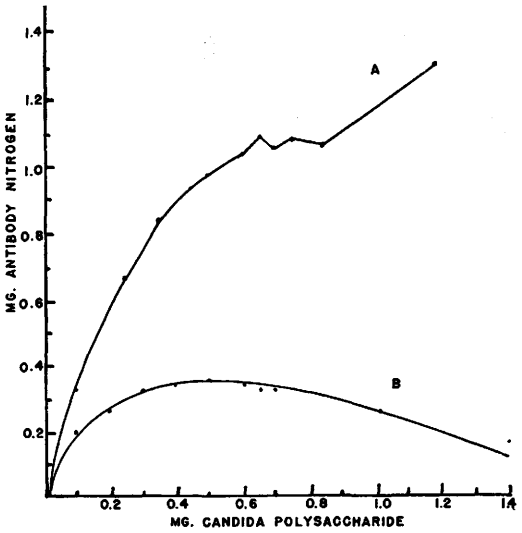

FIG. 1. Reaction of Candida polysaccharide in unabsorbed anticandida rabbit serum (Curve A) and anticandida rabbit serum absorbed with Saccharomyces polysaccharide (Curve B).

To confirm the presence of 2 distinct antibodies in the Candida antiserum, thus reflecting the impurity of the antigen preparation and allowing us to characterize the combining characteristics of the common and type-specific antibody, advantage was taken of the fact that *Saccharomyces cerevisiae* shares a common antigen with the Candida genus(7,8). Thorough absorption of Candida antiserum with Saccharomyces polysaccharide, prepared as described in methods, removed the greater portion of antibody while leaving behind a small fraction which still reacted with the Candida polysaccharide (Fig. 1, Curve B). Note that only after absorption of this common antibody was it possible to obtain a curve consistent with a single antigen-antibody system, showing the formation of soluble complexes and decreasing amounts of antibody precipitated in the region of antigen excess.

Similar experiments were conducted using Saccharomyces antiserum. Absorption of this antiserum with Candida antigen removed a major portion of the antibody and left behind a smaller fraction which still reacted with the Saccharomyces antigen. Thus it appears that rabbit antisera produced by either Candida or

Saccharomyces contain common antibody far in excess of the type-specific antibody.

Discussion. The separation of a type-specific antigen from common group antigen in polysaccharide preparations from *Candida albicans* would offer a distinct advantage when testing for humoral or tissue antibodies in the diagnosis of Moniliasis, since material used at present unavoidably detects antibodies to Saccharomyces as well as Candida. The preparation from *Candida albicans* appeared to contain 2 components, one rapidly soluble in distilled water and the other slowly soluble and gelatinous. While it has not been possible to separate 2 distinct components by chemical means, the rapidly soluble fraction would appear to contain the common antigen since the Saccharomyces polysaccharide preparation containing this common antigen is rapidly soluble in distilled water. Other methods for separating the components are being tried, *i.e.*, electrophoresis, chromotography.

The possibility that autoclaving the antigen accounted for its unusual activity can be discounted since the same pattern of activity was observed in experiments using polysaccharides prepared from saline washings of unheated Candida cells, and from aerated liquid medium cultures. Further evidence of inhomogeneity of the polysaccharide preparation was obtained using the gel diffusion method described by Ouchterlony(9), which has been found effective in characterizing antigens containing components of different specificity. When Candida antigen and Candida antiserum were allowed to diffuse toward one another through a semi-solid gel 2 distinct bands of precipitate were formed, whereas only one was seen when Saccharomyces antigen was substituted for the Candida antigen.

Summary. A polysaccharide preparation derived from autoclaved cells of *Candida albicans* or saline washings of unheated cells has been shown by quantitative immunogolic methods to contain a common antigen shared with *Saccharomyces cerevisiae* and a type-specific Candida polysaccharide antigen. Antiserum obtained from rabbits immunized with heat-killed *Candida albicans* or *Saccharomyces cerevisiae* contains considerably more antibody reacting to the common antigen than to the type-specific antigen.

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