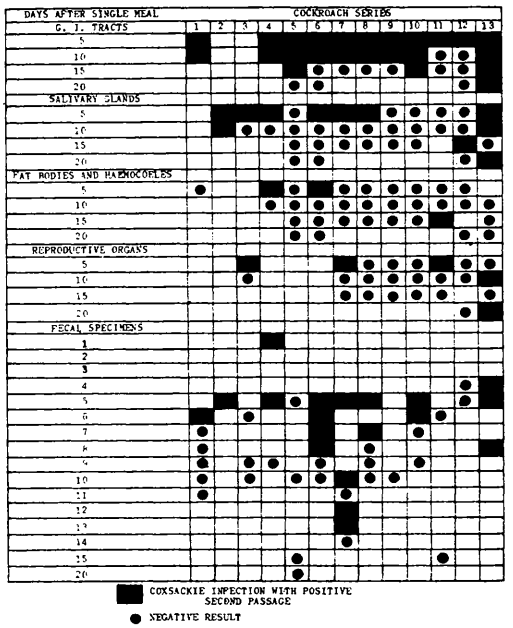
TABLE II. Recovery of Coxsackie Virus from Cockroach Viscera.




sporadic Coxsackie and poliomyelitis infections in man.

*Summary.* Gastrointestinal tracts removed at 5-day intervals for up to 20 days from cockroaches, *Periplaneta americana,* fed a single meal of Coxsackie virus contained sufficient virus to paralyze and kill test mice. Salivary glands removed after 5 days from the test cockroaches also caused paralysis and death in test mice; in one experiment, salivary glands removed 5, 10 and 20 days after a single feeding of virus caused Coxsackie infection in test mice.

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## Heat-Stability of *Candida albicans* and *Saccharomyces cerevisiae* Hemagglutinating Antigens. (23196)

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Erythrocyte sensitizing substances (ESS) derived from *Candida albicans* and *Saccharomyces cerevisiae* have recently been described as requiring lipid for sensitization of red cells in the hemagglutination test(1). Further studies of these antigens led to an investigation of their heat-stability. Unexpectedly it was established that these antigens prepared by similar technics react differently to heat in respect to their capacity subsequently to sensitize red cells. In the following report serologic activity of ESS from *C. albicans* and *S. cerevisiae* is compared to whole yeast-cell agglutinating antigen from these organisms before and after heat-treatment.

*Materials and methods. Preparation of antigens and serologic tests employed.* ESS from *C. albicans* and *S. cerevisiae* derived from liquid medium, and antisera to these organisms were obtained as described recently (1). The hemagglutination test described previously was used to test activity of these antigens(2). Whole yeast-cell antigens were prepared from cells grown at optimal temperatures on Sabouraud's dextrose agar. The cells were heat-killed at 60°C for 2 hours, washed 3 times in saline and adjusted to a No. 2 McFarland nephelometer tube. The yeast-cell agglutination test employed 0.4 ml serial dilutions of inactivated rabbit sera to

TABLE I. Serologic Activity of *Candida albicans* and *Saccharomyces cerevisiae* Antigens before and after Heat Treatment.

| Antigen | Treatment of antigens: Unheated, Antiserum Cand. | Unheated, Antiserum Sacch. | Heated (70°C 48 hr), Antiserum Cand. | Heated (70°C 48 hr), Antiserum Sacch. | Autoclaving, Antiserum Cand. | Autoclaving, Antiserum Sacch. |
|---|---|---|---|---|---|---|
| *Candida albicans* (yeast cells) | 2048* | 128 | 2048 | 128 | | |
| *Saccharomyces cerevisiae* (yeast cells) | 256 | 2048 | 256 | 2048 | | |
| Candida ESS | | | | | | |
| Undil. | 256 | 32 | 512 | 64 | 1024 | 128 |
| 1:2 | 128 | 0 | 256 | 64 | 256 | 128 |
| 1:4 | 32 | 0 | 32 | 0 | 128 | 0 |
| Saccharomyces ESS | | | | | | |
| Undil. | 2048 | 256 | 0 | 0 | 0 | 0 |
| 1:2 | 2048 | 128 | 0 | 0 | 0 | 0 |
| 1:4 | 2048 | 128 | 0 | 0 | 0 | 0 |

* Reciprocal of highest serum dilution giving agglutination.

which were added 0.4 ml antigen. The test was incubated for 2 hours at 37°C and spun at 1000 rpm for 5 minutes. The degree of clumping indicating a positive reaction was read by gently flicking the tubes to resuspend the sediment. *Heat-treatment of antigens.* Antigens submitted to heat-treatment were either placed in a thermostatically controlled water-bath at 70°C or autoclaved at 121°C at 15 lb pressure for 15 minutes. The heated antigens were then titrated in homologous and heterologous antisera to determine any alterations in sensitizing activity.

*Results.* The effects of heating on yeast-cell agglutinating antigens and ESS substances are presented in Table I. Heat did not diminish serologic activity of the yeast-cell agglutinating antigens or ESS from Candida but did have a profound effect on Saccharomyces ESS. The ESS from Saccharomyces antigen was totally inactivated by 70°C for 48 hours or autoclaving while the Candida ESS showed some increase in antigen activity as a result of heating. In experiments conducted to determine rate of inactivation of the Saccharomyces antigen it was found that at 38 hours red cells sensitized with the heated material still agglutinated in homologous antiserum. This residual antibody was blocked by adding small increments of the Candida ESS antigen to the Saccharomyces antiserum, suggesting the common antigen shared by these organisms is more resistant to heat than the type-specific one. *Studies with inactivated Saccharomyces ESS.* As noted previously ESS preparations inactivated by various procedures did not suffer a concomitant loss in precipitin activity, presumably due to stability of the haptenic polysaccharide portion of the antigen(1). In the present heat experiments there was likewise no loss in either qualitative or quantitative precipitin activities of the heated Saccharomyces ESS despite total loss of its capacity to sensitize erythrocytes.

When Saccharomyces and/or Candida antiserum was treated with aliquots of the active or heat-inactivated Saccharomyces ESS to determine whether heating had altered capacity of the antigen to block the hemagglutination test, oddly enough both the heated and unheated materials precipitated large quantities of antibody from either Candida or Saccharomyces antiserum but did not remove or block all antibody which agglutinated red cells sensitized with active Saccharomyces ESS. This residual antibody was blocked by subsequent addition of Candida ESS, which indicates that it was common antibody. Why the Saccharomyces preparation did not remove all common antibody is obscure at this time, however, it is possible that common antigen was almost totally lacking in these ESS preparations.

*Discussion.* The probability is strong in view of recent findings that ESS preparations

from Candida and Saccharomyces organisms as well as from Staphylococcus(3) contain a haptenic polysaccharide and a lipid-like material which is instrumental in effecting attachment of the polysaccharide to red cells. There appears to be further evidence in this direction as a result of heating experiments which show that Saccharomyces ESS antigen can be inactivated without altering its serologic activity as measured in the precipitin reaction. The failure of heat to inactivate the yeast-cell agglutinating antigens can be interpreted to mean that the haptenic polysaccharide, identical to the ESS polysaccharide fraction, is firmly bound to the cell surface and does not need a lipid factor to support serologic activity; it also indicates the heat stability of the haptenic polysaccharide antigen on the surface of the yeast-cell. On the other hand the Saccharomyces ESS, a cell free antigen, requires participation of lipid to develop the antigenic complex involving erythrocytes and haptenic polysaccharide and can be inactivated by heat through the lability of the lipid in this instance.

Oeding(3) has reported on the heat-lability of the Staphylococcal ESS. While it was very resistant to heat, 1 N NaOH or storage led to its inactivation without loss of precipitinogen activity. On the basis of absorption with normal red cells which led to the removal of the sensitizing factor but very little precipitinogen, Oeding suggests the presence of 2 antigens with identical specificity but only one with the ability to sensitize red cells. We have attempted to identify 2 such components in our preparations with similar technics but have found that absorption of extracts with normal red cells, which inactivates their sensitizing properties does not do so by removal of the ESS but by the blocking action of surface protein of normal red cells. The latter can be removed by deproteinization resulting in reactivation of absorbed, inactivated extracts.

It has been stated that heat is not necessary for activation of these ESS preparations (1). In the present study it was found that the Candida extracts, while not requiring heat for activation, did acquire enhanced activity as a result of heat treatment (Table I). Both Neter(4) and Landy(5) have previously noted the activating effect of heat on enterobacterial hemagglutinating substances.

*C. albicans* and *S. cerevisiae* share a common antigen which can be demonstrated by the gel diffusion technic(6). Likewise red cells sensitized with ESS from either organism cross react in the other antiserum which is further evidence of common antigen. While the Saccharomyces ESS activity is totally inactivated by heat for either serum, the Candida ESS is somewhat enhanced by heat in both sera. It is very likely, from these observations, that the common antigen shared by these 2 organisms is combined with different lipid carriers, one heat-labile, the other heat-stable.

These studies were initially undertaken to study the antigens of a common yeast-like pathogen such as *Candida albicans* and a saprophytic yeast *Saccharomyces cerevisiae*. While there are many striking differences already recorded regarding animal pathogenicity and morphologic features of these organisms, it is interesting to note that antigen derived by similar means from these organisms exhibit striking differences in their stability to heat.

*Summary.* The heat-lability of erythrocyte sensitizing substances from *Candida albicans* and *Saccharomyces cerevisiae* was examined. Heat-treatment enhanced activity of Candida (ESS) antigens while it totally inactivated Saccharomyces (ESS) antigen. The common (ESS) antigen shared by these organisms is believed to be more resistant to heat than the type-specific antigen. Heat treatment did not affect activity of the whole yeast-cell antigens of *Candida albicans* or *Saccharomyces cerevisiae*.

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## Antigenicity of Hyaluronic Acid.* (23197)

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Studies of antigenicity of hyaluronic acid indicate that it is not antigenic by itself(1) and when coupled chemically with protein it does not act as a hapten(2). Recent work of Glynn and Holborow(3) reports the production of complete antigens from vegetable polysaccharides (chondroitin sulfate) to autoantigens by group A hemolytic streptococci (4). Although Glynn and Holborow have since indicated that they have been unable to repeat their work and that observed antibodies were directed against blood group like substances and not against chondroitin sulfate itself(5), the question of whether hyaluronic acid, and other tissue polysaccharides might become autoantigenic is important in possible interpretations of the pathogenesis of rheumatic fever. It is the purpose of this paper to report attempts to render hyaluronic acid antigenic.

*Materials.* Hyaluronic acid was prepared from human umbilical cords according to method described by Jeanloz and Forchielli (6), and digested with pepsin but not with trypsin.‡ Trypsin digestion was eliminated to avoid the necessity of lowering the pH with possible denaturation of hyaluronic acid. Remaining proteins were eliminated by Sevag's procedure(7) which was performed at least 10 times. Sulfur-containing compounds were removed by treating the hyaluronic acid solution twice with ammonium sulfate and pyridine(6). Streptococcal hyaluronic acid was prepared from the N.Y. 5 strain of group A hemolytic streptococcus, employing the method of Pierce and White(8). The small amount of residual protein was removed by Sevag's procedure. A crude extract of umbilical cords was prepared according to the method of McClean *et al.*(9). Bacteria used as antigens were 3 strains of group A beta hemolytic streptococci belonging to serological types 1, 4 and 14, and a strain of hemolytic *Staphylococcus aureus*. In preparation of materials for inoculation, the bacteria were incubated for 18 hours at 37°C in 100 ml of brain heart infusion broth, centrifuged and taken up in 25 ml of 0.85% saline. Half of this bacterial suspension was employed as antigen without exposure to hyaluronic acid, and half was centrifuged and resuspended in 10 ml of a solution of cord hyaluronic acid, 1 mg hyaluronic acid/ml of 0.85% saline. Bacteria were not exposed to streptococcal hyaluronic acid. Half of the bacteria were incubated at 37°C for 1 hour with hyaluronic acid *before* being heat-killed and half *after* being heat-killed. Then these suspensions were washed 4 times in 0.85% saline. A portion of the suspension in contact with hyaluronic acid before killing was not washed to test any possible effect of washing on antigenicity. Careful controls were observed to insure pure cultures. The cord hyaluronic acid and streptococcal hyaluronic acid injected without bacteria contained 1 mg and 1.3 mg hyaluronic acid respectively/ml of

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‡ Had trypsin digestion been done it is possible that the numerous attempts to remove proteins by Sevag's procedure might have been unnecessary. It seems likely that each Sevag procedure removed with it some of the hyaluronate, thus accounting for the very small yield of 255 mg from a starting material of 2000 g of human umbilical cords which had been stored in acetone.