of porphyrins formed *in vitro* as compared to those formed *in vivo*. Uroporphyrin-like compounds are formed predominately by preparations of uterine tissue; whereas, almost all porphyrin deposited on shell and found in intact uterine tissue is a protoporphyrin-type compound. Some coproporphyrin, 0.2 γ/g of uterine tissue, is also found.

Summary. 1. Uterine tissue, from white or brown egg-laying hens, forms twice as much porphyrin, *in vitro,* from delta-aminolevulenic acid as liver tissue. Supernatant preparations of uterine tissue form predominately uroporphyrin, small amounts of protoporphyrin, and trace amounts of coproporphyrin from delta-aminolevulenic acid. These compounds were tentatively identified by their behavior in the fractionation procedure used and by their absorption curves. 2. Oöporphyrin and coproporphyrin were extracted from the glandular tissue of the uterus of laying hens.

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Role of Lipid in Hemagglutination by *Candida albicans* and *Saccharomyces cerevisiae.** (22920)

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An erythrocyte sensitizing antigen (ESS) from saline washings of bead-disrupted *Candida albicans* and *Saccharomyces cerevisiae* has recently been described(1). The activity of the antigen was measured in the hemagglutination reaction in which simple admixture of erythrocytes with polysaccharides leads to acquisition of immunologic activity by the red cells as demonstrated by their agglutination in homologous antiserum. In addition to such activity these preparations were noted to possess immunologic precipitating properties while on the basis of positive Molisch tests and negative Biuret tests they were characterized essentially as polysaccharide. Recent observations have shown that occasionally the erythrocyte sensitizing activity of an antigen may disappear spontaneously on standing while its precipitating activity remains unchanged.

In the experiments reported below it is proposed that saline extracts from these organisms, with the overall properties of polysaccharides, contain lipid which is necessary for the sensitization of red cells. The current interest in the pyrogenic, endotoxic and hemagglutinative properties of the lipopolysaccharides from Gram-negative bacteria(2,3) makes the findings reported below of antigenic compounds believed to be lipopolysaccharide isolated from yeast and yeast-like organisms of considerable interest.

Materials and methods. Antigen prepara-

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tions: a. *Solid medium. C. albicans* or *S. cerevisiae* were cultured on Sabouraud's dextrose agar in Roux bottles. The antigen was harvested with isotonic saline and extracted as described in a previous report(1). b. A liquid medium containing 4% dextrose and 1% neopeptone was distributed in gallon jugs and aerated after inoculation with 0.3% by volume of either *C. albicans* or *S. cerevisiae*. Cohen's filtration method(4) was used to separate cells from medium after 48-72 hours incubation. The antigen, produced free in the surrounding medium, was concentrated approximately 30 times by pervaporation or ultrafiltration. It was adjusted to contain 10% sodium acetate, 1% acetic acid and then treated with 2 vol. of 95% alcohol. The resulting precipitate was centrifuged and reconstituted in isotonic saline. *Antisera and immunologic tests.* Cells from liquid cultures were used to immunize white rabbits with either *C. albicans* or *S. cerevisiae* according to an immunization schedule given elsewhere (1). A qualitative precipitin test in which antigen dilutions are layered over undiluted antisera in 6 mm tubes was employed to estimate comparative strength of antigen preparations. A quantitative precipitin test was also employed essentially as described by Kabat and Mayer(5). Antibody nitrogen was determined by a modified biuret technic(1). The hemagglutination test described in a previous report was used to estimate and measure the sensitizing capacity of the antigen preparations(1).

Results. Antigens derived from both liquid and solid mediums exhibited precipitin activity and showed the presence of ESS as measured in the hemagglutination reaction. The antigens differed in appearance and serologic activity. The liquid antigen gave positive ring tests in homologous antiserum in dilutions of 1:1080 as opposed to 1:200 for the solid antigen. However, a 1:8 dilution of solid antigen as opposed to a 1:2 for the liquid antigen was capable of sensitizing red cells in the hemagglutination test. The lack of correlation between the precipitin titers and ESS activity of these antigens is noteworthy.

TABLE I. Removal of ESS from *C. albicans* Antigen by Seitz Filtration and Exhaustive Deproteinization.

Procedure done on antigen	Precipitin test (titer)*		Hemagglutination test (titer)*	
	Before	After	Before	After
Seitz filtration†	1:200	1:800	1:1080	0
Deproteinization‡	1:1080	1:2160	1:128	0

* Precipitin test: highest dilution of antigen giving a positive ring test. Hemagglutination test: highest dilution of antiserum causing hemagglutination of red cells.

† Antigen diluted 1:4 before Seitz and tested undiluted after Seitz.

‡ Antigen concentrated 2-fold after deproteinization.

Removal of ESS by deproteinization procedures. Exhaustive treatment, 6-7 times, of the antigens from either *C. albicans* or *S. cerevisiae* with a chloroform and n-butyl alcohol mixture as required in Sevag's deproteinization technic(6) led to removal of ESS and residual protein but no loss of precipitating activity (Table I). It has not yet been possible to determine whether the ESS is removed at the emulsion interface layer or in the chloroform layer. The possibility of the ESS being protein is slight since Boyden(7) has shown that erythrocytes must be chemically pretreated before they will adsorb proteins preferentially over polysaccharides.

Removal of ESS by Seitz filtration. The ESS was removed from Candida or Saccharomyces antigen preparations when small aliquots were passed through Seitz filters and, as

TABLE II. Quantitative Nitrogen Determinations on Antibody Precipitated from Saccharomyces Rabbit Antiserum by Seitz Filtered Saccharomyces Liquid Antigen.

Amt of antigen (ml)	Antibody N (mg)/ml of antiserum			
	Before Seitz (dil. 1:4)		After Seitz (undil.)	
	(1)*	(2)	(1)	(2)
.02	.064	.063	.112	.113
.05	.160	.144	.184	.192
.10	.192	.244	.208	.280
.20	.208	.304	.209	.304
.30	.208	.304	.184	.280
.40	.184	.280	.160	.256
.50	.160	.259	.160	.256
.60	.160		.144	

* (1) and (2) represent individual tests using different antisera and antigen samples.

TABLE III. Inactivation of ESS from *C. albicans* and *S. cerevisiae* by Lipase as Measured in Hemagglutination Test.

Red blood cells sensitized by *C. albicans* antigen after treatment of latter with			Titer of *C. albicans* antiserum causing hemagglutination
Lipase (0.6 mg/ml) for	0 hr at	37°C	1:4096
	5	37	1:1024
	25	37	1: 512
	72	37	0
	72	70	1:2048
NaCl (control)	72	37	1:4096
	72	70	1:4096

Red blood cells sensitized by *S. cerevisiae* antigen after treatment of latter with			Titer of *S. cerevisiae* antiserum causing hemagglutination
Lipase (0.16 mg/ml) for	0 hr at	37°C	1:4096
	5	37	1: 512
	24	37	0
	24	70	1: 64
NaCl (control)	24	70	1: 64
	24	37	1:4096

noted with deproteinization, no loss was observed in precipitin activity (Table I). As can be seen in Table II, precipitin properties were not quantitatively diminished by Seitz filtration. Furthermore, absorption of either Candida or Saccharomyces antiserum with Seitz-filtered, ESS-negative antigens completely removed antibody agglutinating red cells sensitized with the unfiltered antigens.

Blocking of ESS by addition of albumin. Neter(8) showed that various protein substances inactivate the ESS from *E. coli*. The blocking action of the protein is against the antigen itself and not the red cells. Bovine serum albumin, Fraction V, in 0.2% concentration inactivated the ESS of both Candida and Saccharomyces antigens. Egg albumin was less active but this may be attributable to poorer solubility of egg albumin in saline. Inactivation of the antigen again was not accompanied by a reduction in precipitin activity.

Inactivation of ESS from C. albicans or S. cerevisiae by lipase. The following experiments with lipase indicate that lipid is in some way involved in sensitization of red blood cells. A purified, defatted pancreas preparation was employed. Large quantities of both Saccharomyces and Candida antigens were passed through Seitz filters to assure sterility. This procedure did not affect antigen activity as noted previously when small aliquots were filtered. Pancreatic lipase powder was added to the antigens in concentrations ranging from 0.6 mg per ml to 0.16 mg/ml. Merthiolate was added in a final concentration of 1:50,000. Samples of antigens plus lipase were incubated at 37°C and 70°C, and tested after various intervals for their ability to sensitize red cells.

The results of these tests are given in Table III. Both Candida and Saccharomyces antigens were inactivated by pancreatic lipase. If lipase was added to the antigens before Seitz filtration there was no loss in antigen activity. Likewise no loss, due to lipase activity, was noted if the lipase-antigen mixture was kept at 70°C. Since lipase is inactivated at temperatures above 55°C, and also if passed through a Seitz filter, these data would indicate that lipase acts enzymatically to destroy the sensitizing properties of the antigens. Saccharomyces antigen appeared to be a more suitable substrate for lipase and more heat-labile than the Candida antigen.

Discussion. Keogh, North and Warburton (9) isolated erythrocyte sensitizing antigens from many Gram negative and positive organisms. In their early work they hypothesized that type-specific polysaccharides isolated from these organisms apparently required a second compound in order to adsorb the former on to the surface of red cells. They later felt that polysaccharide antigens which failed to sensitize had undergone chemical degradation and there was no need to postulate a second substance. It was felt by the present investigator that if polysaccharides alone were responsible for modification of the red cell surface in the hemagglutination test, the potential strength of antigens doing this should be predicted by their comparative activity in the qualitative precipitin test, in which polysaccharides readily give positive re-

actions. During the present work with polysaccharides from yeast and yeast-like organisms which were extracted and purified by gentle procedures, or treated as described in this report, it was consistently noted that precipitin activity of the antigens did not parallel their ability to sensitize red cells. It was further noted that there was no difference in quantitative precipitating activity of antigens which are active erythrocyte sensitizers at one stage of handling and then inactivated after purification or other manipulations described in this report. The inactivation of the ESS activity by lipase or by deproteinization with organic solvents would seem to implicate a lipid containing antigen in sensitization of red cells.

Role of lipids in erythrocyte sensitization. In recent months groups of investigators led by Neter(2) and Landy(3) have isolated antigenic lipopolysaccharides from *E. coli* and *S. typhosa* respectively. These materials extracted by Boivin's trichloroacetic acid method or by the phenol water extraction method of Westphal and associates(10), exhibit erythrocyte sensitizing activity, pyrogenic and endotoxic properties. Both have observed that heat or mild alkali treatment of their antigens is necessary before they will sensitize erythrocytes. The release of lipid by this treatment is believed by Landy to have something to do with the activation of the material (2). However, attempts to block the active antigen with the released lipid have been unsuccessful. It is interesting to note that total bound lipids are not removed by heat or alkali but require mineral acid hydrolysis. Unlike the findings of the authors cited, neither heat treatment nor alkali is necessary to activate erythrocyte sensitizing properties of the yeast antigen by removing lipid. On the contrary a lipid fraction appears to be essential for sensitization of the erythrocytes.

Neter *et al.*(11) have shown that lecithin and cholesterol inactivate the *E. coli* antigen when used in extremely small amounts. The inactivation effect is enhanced by boiling the lipid-antigen mixtures. The pancreatic extract used in the present report contained 0.5 mg of cholesterol per gram. However, experiments conducted with pure cholesterol showed no inactivating effect on the yeast antigens when used at concentrations considerably higher than that found in the pancreatic extract. Landy reports(3) that using the enzyme pancreatin in an attempt to hydrolyze bound lipid from his antigen he succeeded in activating its erythrocyte sensitizing properties. However, it was found that activation was not due to enzyme activity but to alkali buffering the enzyme system.

Lipopolysaccharide-protein complexes. Neter(2) described the ability of the lipopolysaccharides from *E. coli* to complex with protein. These complexes are only stable, *i.e.* not precipitated with trichloroacetic acid if the antigen is first treated with alkali or heat. The antigens resembling lipopolysaccharides from yeast or yeast-like organisms also show an affinity for complexing with protein in this highly stable manner without requiring preliminary treatment.

Further investigations are in progress to determine the exact chemical nature of the yeast-like antigens responsible for sensitizing erythrocytes, and to explore the possibility that these fungi contain materials similar to the lipopolysaccharides of Gram negative organisms which are pyrogenic and endotoxic.

Summary. The erythrocyte sensitizing antigen in saline extracts from yeast and yeast-like organisms was inactivated by a number of procedures including exhaustive deproteinization wtih chloroform-n-butyl alcohol mixtures, Seitz filtration, albumin blocking and treatment with lipase. The erythrocyte sensitizing antigen from *S. cerevisiae* was found to be more heat-labile and more readily inactivated by lipase than the *C. albicans* antigen.

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Reticulocytosis Induced by Serum from Hypoxic Animals.* (22921)

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It has been demonstrated that whole plasma and serum from animals with blood loss anemia will induce a reticulocytosis when injected into normal animals(1-5). When large amounts of such plasma are injected over a prolonged period of time, increases in number of red blood cells, in hematocrit and in bone marrow normoblasts have been observed(3). The following experiment was carried out to investigate whether the serum of animals exposed to low oxygen tension of inspired air would induce a similar erythropoietic response.

Methods. To utilize the bioassay technic previously described(3) white New Zealand rabbits, weighing 2-3 kg, were used both as donors and recipients. Normal serum was obtained from normal animals kept at room air, and "hypoxic serum" from normal animals kept in 10% oxygen for 48 hours. The latter animals were kept in a bank of 8 cages, enclosed in an air tight envelope of plastic yard goods sealed around inlet and outflow tubes. Sufficient food and water was placed in the cages to provide for the animals until the end of the hypoxic period. Trays of calcium hydroxide and calcium chloride were used to absorb excess carbon dioxide and water. The air within the envelope was circulated by a small electric fan. Its temperature was checked and never rose above 31°C. The oxygen level in the envelope was kept constant at 10% by admitting compressed room air and pure nitrogen at appropriate flow rates. The total mixed air flow was 0.75-1.0 liter per minute per animal. The actual oxygen level was checked frequently by use of Beckman electromagnetic oxygen analyzer. Four 48 hour runs were made with above equipment with 8-12 animals in each run. After each run 2 animals were kept alive in room air as "tracers." The others were bled to death by intracardiac puncture within 1½ hours after end of hypoxic exposure. The "hypoxic serum" so obtained was pooled and frozen or refrigerated until used. Normal serum was obtained and stored in a similar fashion. Normal recipient rabbits each received 50 ml of serum i.v. once a day for 4 days. Daily hemoglobin determinations and reticulocyte counts were carried out on recipient animals and on tracer animals from the hypoxic group of donors. Reticulocyte counts were made on cover glass smears prestained with brilliant cresyl blue and counterstained with Wright's stain. Reticulocyte smears were counted "blind" and a minimum of 1000 red cells was examined—except on days 2, 3, 4, 5 when a minimum of 2000 cells was examined.

Results. Tracer animals. (Fig. 1). Eight rabbits, 2 from each of 4 runs, were followed after exposure to 10% oxygen for 48 hours. Although there was a wide variation in response, the mean reticulocyte level rose from a base line of 1.9% to maximum of 6.7% on day 3. Hemoglobin levels remained unchanged. *Normal recipients of normal serum.* (Fig. 2). Six normal rabbits received 200 ml of serum from normal donors. The reticulocyte levels rose slightly from a mean base

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