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Yeast Extracts to Overcome Depressant Effects of Germicide on Skin Respiration.

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It is well known that the use of bactericidal agents in wounds is hampered by the adverse effects of these agents upon the tissues. For example, Bisgard and Baker "believe that there is ample experimental and clinical evidence that the chemical bactericidal agents, which are so generally used to 'disinfect' wounds, destroy or devitalize the tissue cells with which they come in contact and thereby seriously impede the inflammatory response and impair the effectiveness of the natural mechanism of defense."[1]

It is obvious that an agent which would, even in part, offset the deleterious effects of bactericides on tissues with preservation of adequate bactericidal powers might be of clinical value.

In the course of studies on the effects of yeast and tissue extracts on cellular metabolism we observed that certain yeast extracts antagonized the toxicity of phenylmercuric nitrate and *n*-butyl-*p*-hydroxybenzoate for molds.[2] It appeared to us that such extracts, and particularly certain fractions known to increase tissue respiration,[3] might serve to protect the tissues from the toxic action of germicides.

As a measure of toxicity to tissues we used the respiratory-depressing effect of the germicide since respiration is a satisfactory index of tissue vitality[4] and since a number of workers have proposed a manometric method of germicide evaluation based upon the correlation of the toxic effects of germicides with their respiratory-depressant activities on organism and host tissue.[5] In line

1 Bisgard, J. D., and Baker, C. P., *Surg. Gynecol. Obstet.*, 1942, **74**, 20.

2 Cook, E. S., and Kreke, C. W., *Nature*, 1940, **146**, 688.

3 Cook, E. S., Kreke, C. W., and Nutini, L. G., *Studies Inst. Divi Thomae*, 1938, **2**, 23.

4 Adams, P. D., *Arch. Dermatol. and Syphilol.*, 1936, **36**, 606; Cook, E. S., *Chem. Products*, 1939, **1**, 65, and 1939, **2**, 89; Amersbach, J. C., Nutini, L. G., and Cook, E. S., *Arch. Dermatol. Syphilol.*, 1941, **43**, 949; Manifold, M. C., *Proc. Roy. Soc. Med.*, 1940, **33**, 12.

5 Bronfenbrenner, J., Hershey, A. D., and Doubly, J., *J. Bact.*, 1939, **37**, 583; Ely, J. D., *Ibid.*, 1939, **38**, 391; Greig, M. E., and Hoogerheide, J. C., *Ibid.*, 1941, **41**, 549, 557; Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **73**, 249.

with these findings, we had also noted that, with certain fractions from yeast, ability to stimulate yeast respiration paralleled ability to increase yeast proliferation.[6]

The experiments reported in the present paper concern the toxic effects of phenylmercuric nitrate on rat skin as observed by manometric determination of skin respiration, the overcoming of these toxic effects by the addition of a yeast extract, and a comparison of the germicidal efficiency of the drug on *Staphylococcus aureus* with and without the addition of the extract.

Respiration measurements were made by the previously described[3] Warburg method on the abdominal skin of female albino rats approximately one year old. Determinations were made at 37.5°C in Ringer-phosphate-glucose solution (0.2% glucose, pH 7.2). Aqueous-alcoholic yeast extracts were prepared as described previously[3] from Fleischmann's bakers' yeast and corresponded to Fraction A of the earlier work.[3] A stock culture of *S. aureus* obtained from our bacteriology laboratory was grown in nutrient broth. Commercial basic phenylmercuric nitrate (Merphenyl Nitrate, Hamilton Laboratories) was employed.

Fig. 1 shows a typical experiment on the effects of phenylmercuric nitrate and of a crude yeast extract upon the respiration of rat skin. Each point represents the average of 3 determinations, which agreed within 10%, on skin from the same animal. Curve I represents the respiration of rat skin under the experimental conditions. In Curve II skin and phenylmercuric nitrate (1:100,000 final concentration) were present at the beginning of the experiment. The toxic effects of the germicide are evident, and at the end of 60 minutes the skin was respiring 24% below the control. At this time, yeast extract was tipped from the side arm to give a concentration of 10 mg of solids per cc (1%). An increase in respiration rate is apparent and the slope of the remainder of Curve II approaches that of Curve I. In Curve III skin and yeast extracts were present at the beginning of the experiment. At the end of 60 minutes a 48% stimulation of skin respiration exists. Addition of phenylmercuric nitrate at this time reduces the respiratory rate to that of the control, with the net result that the respiratory depressant effect of the germicide is offset.

In another set of 3 determinations, doubling the concentration of germicide (1:50,000) doubled the inhibition of skin respiration (46% inhibition as compared with 24% above). Addition of 1% of yeast extract at the end of 60 minutes was ineffective in off-

[6] Cook, E. S., Hart, M. J., and Stimson, M. M., *Biochem. J.*, 1940, **34**, 1580.

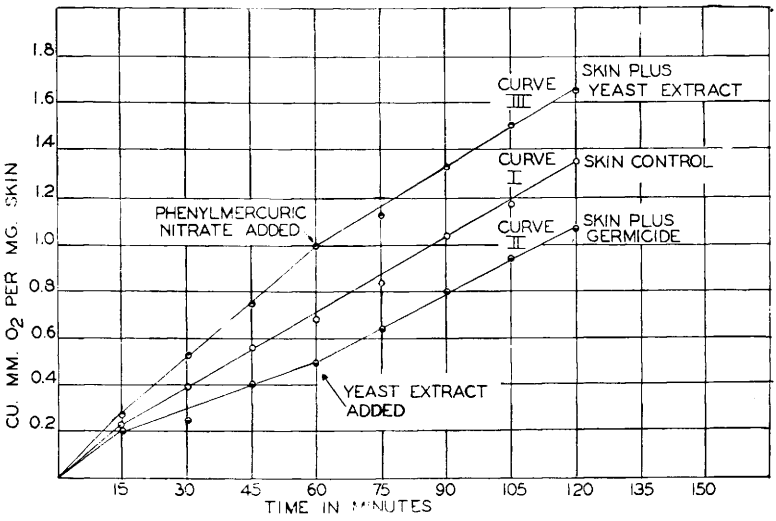

FIG. 1.
Effects of phenylmercuric nitrate and yeast extract on respiration of rat skin.

setting the germicidal depression, but the addition of the yeast extract one hour prior to the addition of the germicide reduced the inhibitory effect of the latter from 46% to 20%. Thus, this concentration of yeast extract did not overcome the greater depression previously caused by a higher concentration of germicide but it did afford protection when added in advance of the germicide. Addition of a mixture of germicide (1:100,000) and yeast extract (1%) after 60 minutes had no effect on the respiratory rate, suggesting either that the inactivation occurs by some sort of combination between the active substances or that the active ingredient of the extract reaches the respiratory systems of the cell at least equally as readily as the germicide.

Repetition of the experiments with different yeast extracts gave results which accorded qualitatively with the above and differed only in amounts of stimulation or depression. This would be expected with the use of unstandardized preparations. A total of 38 determinations was made.

Entirely similar experiments on the respiratory antagonism between phenylmercuric nitrate and yeast extract have been carried out using yeast in place of skin, and the relation between respiratory depression and toxicity has been developed by means of methylene blue staining and parallel experiments on the effects of germicide and yeast extract on growth of yeast in liquid and solid media. These experiments will be reported elsewhere.

In order to see whether the overcoming of toxicity of phenyl-

mercuric nitrate for skin by the yeast extract was accompanied by a loss of toxicity for a typical infectious organism, the following experiment was performed in duplicate. Two sets of tubes each containing 10 cc of nutrient broth were inoculated with a loop of a 3-day broth culture of *Staphylococcus aureus*. In one set of tubes were incorporated dilutions of phenylmercuric nitrate and in the other were incorporated the same dilutions of phenylmercuric nitrate plus 1% (by weight) of yeast extract. After 24 hours' incubation at 37.5°C the amount of growth was determined by centrifugation in Hopkins tubes. Results of a typical experiment are shown in Table I. The yeast extract used for this experiment was the same as that employed in the respiration experiment of Fig. 1. It is readily seen that the yeast extract increases the growth of *S. aureus* in broth and allows growth in dilutions of phenylmercuric nitrate up to 1:1,000,000 which are otherwise germicidal. However, in dilutions of germicide of 1:500,000 or less no growth occurs even in the presence of 1% of yeast extract. Similar results were obtained when *S. aureus* was grown on nutrient agar and the amount of growth was determined by planimeter measurements of the colonies. Results on the solid medium showed somewhat greater variation than those obtained in broth, the latter checking almost exactly.

From the experimental results above it is evident that the toxicity for skin of dilutions of phenylmercuric nitrate as low as 1:100,000 can be offset by 1% of yeast extract. Such dilutions of germicide in the presence of 1% of yeast extract are still completely effective against *S. aureus*. Thus, it should be possible to maintain germicidal efficiency while minimizing toxic action on the host tissue. Preliminary experiments have indicated that combinations of phenylmercuric nitrate and yeast extract also retain germicidal power against *E. coli* and a hemolytic strain of *S. aureus* when tested by the cup plate method, and similar combinations permit the growth of chick epithelium in tissue culture. An extension of these studies

TABLE I.
Effect of Phenylmercuric Nitrate and Yeast Extract on Growth of *S. aureus*.

Dilution of phenylmercuric nitrate	Growth in cc sediment	
	No extract	1% extract
1:200,000	0	0
1:300,000	0	0
1:400,000	0	0
1:500,000	0	0
1:1,000,000	0	.001
1:2,000,000	0	.001
No phenylmercuric nitrate	.005	.007

together with clinical investigations are now in progress.

Summary. One percent of an aqueous-alcoholic yeast extract protects rat skin against the toxic effects of phenylmercuric nitrate in a dilution of 1:100,000 as determined by respiration measurements. A similar amount of yeast extract does not impair the germicidal efficiency of phenylmercuric nitrate against *S. aureus* in dilutions as low as 1:500,000 under the test conditions. Protection of host tissue against toxic action of germicides with maintenance of germicidal properties is thus suggested.

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Failure of Yellow O.B. to Produce Neoplasms.

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Both butter-yellow (*p*-dimethylaminoazobenzene) and *o*-aminoazotoluene are known to be highly effective in producing liver cancer in rats.[1] Since the former dye has been used to color oils, oleomargarine, and other vegetable fat substitutes for butter, and Yellow O.B. (1-*o*-tolylazo-2-naphthylamine), and Yellow A.B. (1-phenylazo-2-naphthylamine), oil-soluble coal tar dyes are permitted in this country to color foodstuffs, the possibility of their acting in an analogous manner to butter-yellow cannot be overlooked as these substances are somewhat related in structure to butter-yellow and *o*-aminoazotoluene (Fig. 1).

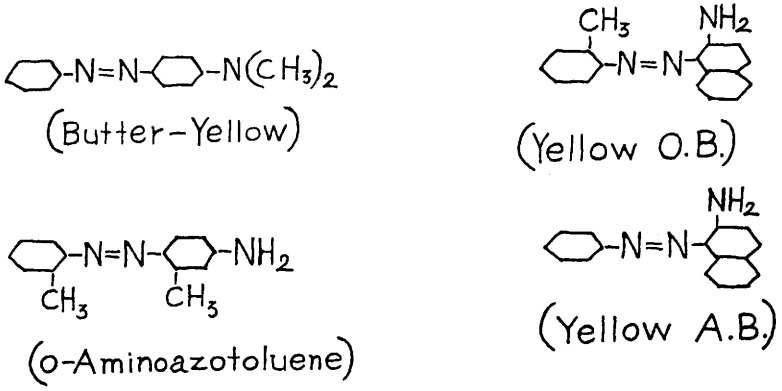

FIG. 1.

1 Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 3.