DISCUSSION

For a long time it has been a mystery to physiologists why digestive juices of the stomach ordinarily do not digest the tissue which goes to form the alimentary canal while ingested tissue is readily digested. Driver and Murlin[2] showed that certain substances can increase the penetrability of intestinal mucosa by a protein molecule, insulin, as judged by its effect on blood sugar. Of these compounds, all that have been tried, namely, calgon, hexylresorcinol, pinacol and methyl salicylate, brought about a tremendous increase in the number and severity of ulcers.

Calgon is a compound noted for its ability to tie up calcium ions.[3] Since this ion has been reported to inhibit absorption[4,5] and since calgon was found to promote the absorption of insulin,[2] it seemed reasonable to expect an increased penetrability by pepsin with a consequent incidence of ulcers when calcium was rendered non-ionic and therefore non-absorbable in the intestine. Calcium combines with lipids and proteins to produce a hardening and protective effect on the mucosa. It is not inconceivable that the beneficial effects of milk in the treatment of ulcers may be attributed in part to its calcium content, and it would seem that fortification of milk with a calcium salt is indicated.

Hexylresorcinol is a powerful surface tension lowering agent and produces structural changes in the membranes of the mucosa which modify permeability.[2,6] Methyl salicylate exerts a special irritating influence on mucous membranes which increases permeation of protein molecules. Pinacol has little irritating effect and does not lower surface tension appreciably. This compound probably changes the penetrability of the mucosa by proteins by its particular molecular configuration, the hydrophobic and hydrophilic groups acting as a lock and key mechanism. The formation of ulcers in these experiments was not due to a difference in pH of the solutions. We conclude that substances which increase the permeability of intestinal mucosa by a lowering of surface tension or other means facilitate the formation of ulcers.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ON THE STAINING OF YEAST SPORES

ALTHOUGH it may be unnecessary to resort to staining procedures to demonstrate spores in some cultures of yeasts, frequently such stains are desirable for proof of the presence of spores or the proper delineation of these. As no one method of staining is used universally, each investigator uses the method of his choice, and this often is one of the methods used for bacterial spores. Thus, Henrici,[1] in a comprehensive review of the cytology and taxonomy of the yeasts, recommends the familiar technique of Moeller. This consists of primary staining with steaming carbol fuchsin, and, after proper destaining, counterstaining with Loeffler's methylene blue.

The above method, as used by the author, often gives slides with considerable precipitate, and frequently color differentiation between spore and vegetative cell is not clear. It seemed possible that the malachite green technique of Schaeffer and Fulton[2] might be more satisfactory since one step, the destaining procedure, is unnecessary. In this technique washing with water is sufficient to remove the primary stain from the vegetative cell. Only a few trials were needed to verify this assumption. Exceptionally clear differentiation was the usual result. Review of the literature revealed that Shimwell[3] had tried the technique with yeasts, although he modified the procedure by using alcohol as a destaining agent. Our results have not indicated the need for this.

The use of 5 per cent. malachite green as suggested by Schaeffer and Fulton for bacterial spores proves somewhat expensive if large numbers of slides are to be stained, as for example, in student laboratory exercises. It is possible to lower the concentration of the dye, and thus reduce the cost of the stain. This and other modifications have produced a staining technique with which we have had uniformly good results with spores of various yeasts. The brilliant contrast between the color of the spores and vegetative cells permits rapid identification, and is so striking that the novice experiences no difficulty in interpretation. In this laboratory, it has been much more successful than the technique of Gray[4] which employs a mixture of malachite green and basic fuchsin.

A number of modifications of the details of the Schaeffer and Fulton formula and procedure have been tested and the formula given below seems to be

[3] B. H. Gilmore, *Ind. and Eng. Chem.*, 29: 584, 1937.
[4] E. Gellhorn and A. Skupa, *Am. Jour. Phys.*, 106: 318, 1933.
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[6] R. Höber, M. Andersh, J. Höber and B. Nebel, *Jour. Cell. Comp. Physiol.*, 13: 195, 1939.
[3] J. L. Shimwell, *Jour. Inst. Brewing*, 44: 474, 1938.
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optimum. Considerable variation in the application of the solutions is possible, and may be necessary with individual cultures, but it is believed that the method suggested will fail only in rare instances.

The solutions needed are as follows: (1) 1 per cent. malachite green dissolved, without heating, in 1 per cent. phenol, and (2) 0.5 per cent. aqueous safranin. The technique suggested for yeast spores is as follows: prepare smear on clean slide, air dry and fix lightly with heat of a Bunsen flame. Cover entire slide with malachite green solution and steam (do not boil) with a low Bunsen flame for two minutes. Wash in a gentle stream of tap water for one minute. Counterstain for 30 seconds with aqueous safranin. Yeast spores, upon examination, appear green, while the asci are a deep pink. With this method, less satisfactory results are obtained with bacterial spores, but further studies are in progress with reference to this question.

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ENZYMATIC CLEANING OF BERKEFELD CANDLES USED IN THE FILTRATION OF HUMAN BLOOD PLASMA

IN instances where filtration was desired in the treatment of plasma it was observed that the pores of new filter candles would clog after slight use and would rarely recover their initial filtering capacities after cleaning and sterilization. Even though preliminary filtration processes were employed the filtering capacities of the candles continued to be impaired.

The filtration was accomplished by pressure in a stainless-steel pressure-chamber having a 10-liter capacity employing 8″ × 2″ Berkefeld candles. New Berkefeld "V" candles would filter about 6 liters of plasma before clogging. And new Berkefeld "W" candles would clog after 1½ to 2 liters of plasma passed through them.

The candles were treated by the following methods:

(A) 1. Backwashing with saline (0.85 per cent.) followed by a thorough scrubbing with a moderately soft brush. 2. Further backwashing with tap water while gently brushing the candle. 3. Boiling the candle in 2 per cent. washing soda for 30 minutes. 4. Boiling in tap water 30 minutes, changing water several times. 5. Cooling and brushing gently under tap water. 6. Backwashing with water. 7. Drying by suction for 10 minutes followed by exposure in a 100° C oven. 8. Sterilization in dry wall oven or autoclave.

(B) 1. Backwashing with saline (0.85 per cent.) followed by a thorough scrubbing with a moderately soft brush. 2. Further backwashing with tap water. 3. Boiling ½ hour in 1 per cent. sodium bicarbonate. 4. Boiling in two changes of distilled water. 5. Cooling and brushing gently under tap water. 6. Drying by suction for 10 minutes followed by exposure in a 100° C oven. 7. Sterilization in dry wall oven or autoclave.

After processing the Berkefeld candles by either of the above methods the "V" candles would only filter about 1 liter and the "W" candles about ½ liter before clogging.

To meet the existing problem the following technique of pepsin digestion was employed in the cleaning of the filter candles and has proven itself to be very satisfactory: Candles were backwashed with saline, followed by a thorough scrubbing with a moderately soft brush while flushing with tap water. The remaining water was blown out by compressed air and the candles submerged in a porcelain container[1] in which 0.5 per cent. pepsin (U.S.P. XI) had been dissolved in aqueous 1 per cent. hydrochloric acid (C.P.). The candles covered by the acidulated pepsin solution were incubated overnight at 37° C, thoroughly backwashed with water while being scrubbed with a moderately soft brush, the water blown out by compressed air, wrapped in heavy paper and sterilized in a dry wall oven or autoclave.

The "V" and "W" Berkefeld candles thus treated were capable of efficiently filtering 8 to 10 liters of human plasma using the positive pressure apparatus. The rate of flow of the "V" candle (one liter every 10 minutes) was approximately twice that of the "W" candle (one liter every 20 minutes).

SUMMARY

Berkefeld candles clogged by human blood plasma were cleared by enzymatic action. Candles incubated in acidulated aqueous pepsin solution, thoroughly rinsed in water, dried and sterilized were capable of efficiently filtering 8 to 10 liters of human blood plasma.

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[1] Vegetable pan from electric refrigerator was employed.

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