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 $8'' \times 2''$ Berkefeld candles. New Berkefeld "V" candles would filter about 6 liters of plasma before clogging. And new Berkefeld "W" candles would clog after $1\frac{1}{2}$ 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 $\frac{1}{2}$ 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 1 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 container¹ 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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