as 3 lenses may grow in the same environment. However, between 25 and 30 days after lensectomy the regenerating lens demonstrates clearly that at this time it becomes linked with a factor, as in the case of a normal fully grown lens, which acts as an inhibitor for lens regeneration from the iris within the eye. This is at a time when the newly forming lens detaches itself from its source of origin, and probably marks a definite period in its differentiation. Both implantations of the detached lens and implantations of various types of dorsal iris emphasize this point.

The results of the experiments carried out within the eye environment seem to be significant. So far it has been found that the dorsal iris grafts of the adult eye of this species only rarely made a feeble but aborted attempt to regenerate a lens when placed outside of the eye environment such as under the skin or within the body cavity. However, this inhibiting effect is possibly of a different nature than that exerted by the lens itself.

14322

Concentration and Preservation of Crude Penicillin.

TSUN T'UNG. (Introduced by Martin Frobisher, Jr.)

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Rapid advances in methods for the purification of penicillin seem to have rendered the small-scale laboratory production of the crude material undesirable. However, since the processing of pure penicillin is complicated and the yield is very small, general clinical trials and intensive laboratory investigations of this substance will not be possible for some time to come. Therefore, the crude penicillin is still useful, provided its bulk can be reduced without loss of potency. The present communication presents a simple and inexpensive procedure for concentrating and preserving crude penicillin for practical use.

The Fleming strain of *Penicillium notatum*[1] was grown on modified Czapek-Dok medium[2] contained in Blake bottles. In order to minimize precipitation of the constituents of the medium, brown sugar and ferrous sulfate were separately sterilized by autoclaving and filtration through Seitz pad, respectively, and were then added to the basic medium.

After one week of incubation at room temperature the penicillin titer of each bottle was determined by the serial dilution method. Meat infusion broth with 1% neopeptone at pH 7.4 was used as diluent. A strain of Lancefield Group A beta hemolytic streptococci was chosen at random as the test organism. Each tube of diluted penicillin was seeded with a drop of an 18-hour broth culture containing about 30 million organisms.

Cultures having effective titers in a dilution of 320 or over were pooled. The pH was adjusted to 6 and 500 ml were poured into a 700 ml distilling flask through cheesecloth. The flask was partly immersed in a water bath the temperature of which was maintained at 60°C. The substrate containing penicillin was evaporated under reduced pressure. The process is illustrated by the accompanying diagram (Fig. 1). A vacuum of about 0.001 mm of mercury was created by a running water pump or the Cenco-Hyvac pump. In 3 hours, the volume of 500 ml could be reduced to 20 ml. The pH of the concentrated stuff was readjusted to 6 and it was sterilized by filtration. While the volume of the crude penicillin was reduced, the titer of the active principle increased manyfold.

The concentrated penicillin was kept in celluloid tubes. Each lot was divided into two portions; one was placed in a dry-ice

1 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

2 Hobby, Gladys, and Myer, Karl, PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 227.

cabinet at a temperature of −76°C[3] and the other in an ordinary ice box at 5°C. Determinations of the titer of the preserved material against the same organism, using the same medium as previously, were made from time to time. Titrations of potency were per-

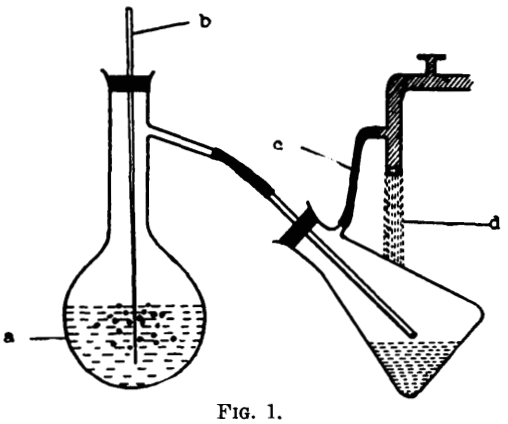

FIG. 1.

Partial Vacuum Concentration Apparatus.
a. Crude culture of *Penicillium notatum*.
b. Tube with capillary tip to produce ebullition.
c. Tube to vacuum pump.
d. Stream of cool water to condense vapor.

formed on the first three consecutive days of storage to insure the accuracy of the initial titer. The keeping qualities of 3 lots of crude penicillin of different titers at the two temperatures are presented in Fig. 2.

At a temperature of −76°C the potency of crude penicillin could be maintained for about one month without marked deterioration. After one to 2 months titers dropped about 50% but remained stable for at least 3 to 5 months thereafter. The same substance kept at 5°C lost its potency rapidly and almost entirely toward the end of 3 months. This seems not to be in accordance with the observations made by Abraham *et al.*[4] and Clutterbuck *et al.*[5] The latter could maintain the potency of a crude penicillin filtrate for 3 months at 0°C and the former stated that in aqueous solution the purified penicillin at 2°C is stable for several months. Probably constituents of the medium and other impurities after being concentrated are responsible for this difference. As facilities for maintaining these temperatures are easily available in ordinary laboratories, the keeping quality of the concentrated crude penicillin at other temperatures was not attempted.

The importance of the adjustment of pH of crude chrysogenin to between 5 and 6 before concentration to render the substance stable during evaporation has been stressed by Clutterbuck.[5] The same pH range is essential for the concentration of penicillin. The pH also plays important roles in the process of concentration and the determination of the titer of penicillin. When the pH was above 6 the culture fluid, when heated, foamed so much that the process of concentration had to be discontinued at once. In titrating a given lot of penicillin the use of a diluent of pH 7.2 may result in a titer twice as high as that obtained with a diluent having a pH 7.6.

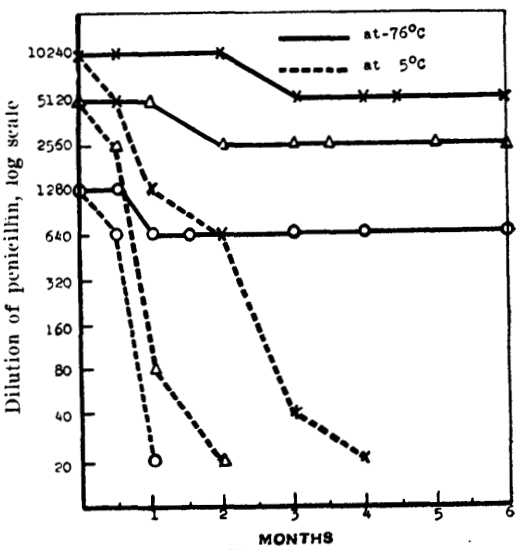

FIG. 2.

Keeping quality of concentrated penicillin at different temperatures.

As is true of spirochaetes and viruses[6] frequent freezing and thawing does not have any appreciable deleterious effect on the preserved crude penicillin. This property permits a convenient and repeated use of the preserved stuff with maintenance of potency for at least 6 months.

[3] Horsfall, Frank K., Jr., *J. Bact.*, 1940, **40**, 559.

[4] Abraham, E. P., and Chain, E., *Brit. J. Exp. Path.*, 1942, **23**, 103.

[5] Clutterbuck, P. W., Lovell, Reginald, and Raistrick, Harold, *Biochem. J.*, 1932, **26**, 1907.

[6] Turner, Thomas B., *J. Exp. Med.*, 1938, **67**, 61.

Summary. The volume of crude penicillin can be greatly reduced with considerable increase in potency of the active principle by a process of low temperature-partial vacuum concentration. After an initial drop the titer of the concentrated material kept in a dry-ice cabinet is maintained for as long as 6 months.

14323

Synergistic Action of Thiourea and Guanidine with Sulfathiazole.

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An advantageous co-action between sulfonamides and urea has been found to exist, both in wounds,[1] and *in vitro*.[2] The conclusion reached by Tsuchiya *et al.* in the latter case have been contested by Kirby.[3] In this paper we have confirmed the finding of Tsuchiya *et al.*, and have extended the observations to include a study of thiourea and iminourea (guanidine). The structural similarity of these two compounds to urea is obvious from the formulæ:

* The authors wish to acknowledge the assistance of Miss Isabel Perrine.

1 Holder, H. G., and MacKay, E. M., *Military Surg.*, 1942, **90**, 509.

2 Tsuchiya, H. M., Tenenberg, D. J., Clark, W. G., and Strakosch, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 247. See also previous papers.

3 Kirby, W. M. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 109.

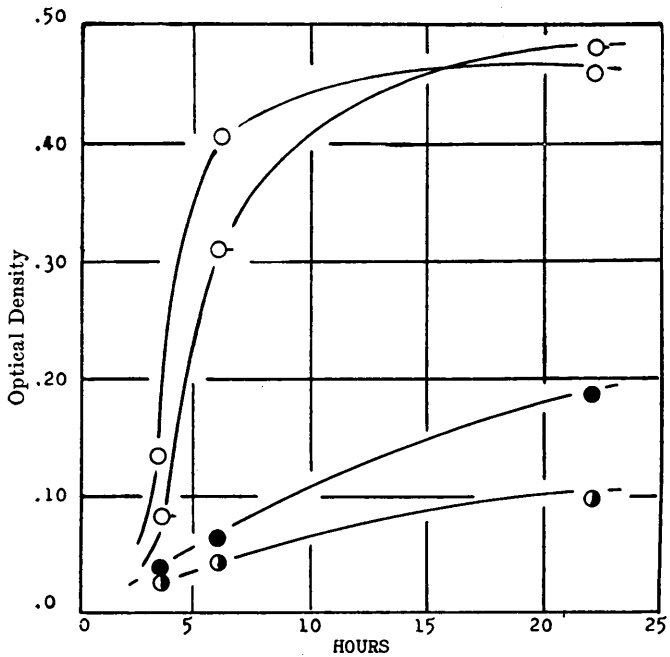

FIG. 1. TIME-GROWTH CURVES OF E. COLI, AS INFLUENCED BY UREA, SODIUM SULFADIAZINE AND THEIR COMBINATION.
○ = Control.
○- = Urea, 1.6% or 0.28 M.
● = Sodium Sulfadiazine, 1 mg. %.
◑ = Urea, 1.6% or 0.28 M , and sodium sulfadiazine, 1 mg.%.