diagrams, (2) cut into small squares it can be used as coverslips, (3) dissolved in acetone or alcohol and ether, or amyl acetate, it forms an excellent substitute for collodion, and can also be used for cementing glass. Serviceable cellulose caps for bottles can be made from solution of old *x*-ray films. As further examples of the utilization of waste material or substitute materials the following are cited :—

1. Cotton-wool plugs, etc., can be salvaged and can often be utilized at least once again.

2. Instead of using brown paper for wrapping laboratory apparatus for sterilization, old newspapers which cost but a fraction of the price of brown paper are satisfactory for this purpose.

3. For filtration of coarse material, as for example before filtration through candle filters or asbestos discs, the material is passed through a column of fine quality sand instead of Kieselguhr.

4. As a substitute for cedar-wood oil for microscopy liquid paraffin is used by many workers. It is not often realized that there are many oils (locally available and at small cost) which can be used. Of these ground-nut oil can be used except where very critical definition is required. Hydnocarpus oil gives better definition than many samples of liquid paraffin.

5. Agar agar—The price of agar has increased considerably since the beginning of the war. Whereas the price was Rs. 3 per pound before the war, it is now Rs. 9-8 a pound for very inferior quality agar. It is possible to salvage a considerable amount of agar from used plates, sterilize it and use it again for ordinary routine purposes. Such agar is satisfactory for the growth of the less fastidious bacteria.

6. Instead of using flasks (which are considerably more expensive now than before the war) bottles of appropriate size can be used. A number of such bottles can often be found amongst the empty bottles that accumulate in a laboratory or in the central stores of a large institution. Bottles of good quality glass are manufactured locally and can be purchased at reasonable prices. The quality of the glass can be easily tested by standard methods (*British Pharmacopœia* : Appendix). Bottles have been introduced for the preparation and storage of media and have proved entirely satisfactory. They stand sterilization well.

7. Instead of wooden packing cases for small specimens sections of bamboo can be used. The bamboo cases are so cut that one side is closed by the natural joint and the other side is closed with a cork or a piece of pith and paper reinforced with wire.

The above are some examples of the many ways in which economy can be effected without loss of efficiency. Such examples can be multiplied many times and will suggest themselves to laboratory workers. These are cited in the hope that they will stimulate workers to develop

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THE STERILITY OF SNAKE VENOM SOLUTIONS

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THE hæmostatic effect of viperine venom and the analgesic effect produced by cobra venom have made venom therapy a popular form of treatment, particularly in chronic painful conditions. In addition to these specific indications snake venoms are in considerable vogue in some centres and are administered in an increasing number of clinical conditions. The venom preparations are used differently, (*a*) by direct local application, or (*b*) by intradermal, subcutaneous, or even intravenous injections.

A severe reaction after a subcutaneous injection of snake venom led to the examination of the venom solution that had been used. This solution contained many viable bacteria. A further 24 samples of venom solutions were examined for sterility. Standard methods of testing were employed, using sufficient fluid medium to ensure that the preservative content of the final dilution shall be less than 0.01 per cent. For the purposes of this calculation the maximum content of the preservative was taken to be 0.5 per cent. Each venom was examined for the presence of aerobic and anaerobic bacteria. The results are summarized below :—

Venom	Number examined	Number passed sterility test	Number not passed sterility test
Cobra ..	11	9	2
Viper ..	14	8	6

The crude venoms when collected are grossly contaminated and the difficulty of obtaining sterile solutions of venoms are well known to all responsible for the preparation of such solutions. Many methods have been advocated for the preparation of sterile solutions. The most satisfactory solutions (as far as the sterility

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such alternative methods and to report them. It is the simple and obvious alternatives that are so valuable and are so often missed.

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MAINTENANCE OF BACTERIAL CULTURES

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IN most laboratories in the North American Continent and in England, the older methods of maintaining bacterial cultures by constant subculturing in suitable media have of recent years been largely superseded by the method of rapid freezing and dehydration *in vacuo*. As early as 1896, Martin suggested the possibility and procedure involved in the preservation of guinea-pig complement in the dried state. Noguchi, in 1907, dried complement and amboceptor on filter paper. Shackell, in 1909, dried complement in the frozen state and showed that it could be preserved for months. Karsner and Collins, in 1919, applied Shackell's method for the preservation of various sera and, in 1923, Hartley and his associates reported the dehydration of complement by distilling off the H_2O *in vacuo*. In 1931, Craigie dried complement in the frozen state by means of a vacuum desiccator and more recently Elser and his co-worker (1936) showed conclusively that biological materials could be dehydrated from the frozen state. This method was admirably developed by Flosdorf and Mudd (1935) in the University of Pennsylvania and resulted in their dry ice 'lyophile apparatus' by means of which many biological materials could be preserved for several months without any detectable deterioration. The same investigators, in a later communication, in 1938, described a new process where the condensation of water vapour in a low temperature bath (dry-ice methyl cellosolve mixture) is replaced by the use of an inexpensive chemical desiccant (the Cryochem process). The method has been very profitably employed for the preservation of various anti-toxins, guinea-pig complement, human plasma for purposes of transfusion, normal and convalescent human sera, enzymes, viruses, bacteria and miscellaneous proteins.

The procedure and apparatus to be described in this communication are a modification of the 'Cryochem process' described by Flosdorf and Mudd in 1938. This has been successfully employed in the preservation of bacterial cultures though it can be used for preservation of sera, protein solutions and other labile biological materials with equal success.

The apparatus

A diagram of the apparatus is shown. The containers used are a number of 1 c.cm. ampoules which are attached to pressure tubing. Any number of containers can be attached by using a multiplicity of glass Y-tube connections. The attachment of the containers leads to two glass cylinders containing either fresh or freshly regenerated anhydrous calcium sulphate (known under the trade-name drierite). Drierite is a comparatively low cost chemical with a powerful affinity for water and is capable of producing a low aqueous tension very rapidly. Also it has no vapour pressure of volatile substances and can cause no harmful effects on labile materials such as has been reported with H_2SO_4 or acid phosphoric anhydride. An additional advantage is that used calcium sulphate can be regenerated for further use by heating for about 6 hours in an oven at 150°C. to 250°C. (180°C. is optimal). The glass cylinders containing drierite are connected to a mercury manometer and a Cenco-Hyvac pump.

The operation of the Cryochem apparatus

The apparatus should first be tested for vacuum tightness with the outlets shut off.

Sterile skimmed milk has been found to be the best medium in which to suspend the bacteria. Overnight cultures of organisms are made and the growth is made into a semi-solid emulsion by adding a small amount of milk. Work during the whole operation should be carried out under sterile conditions lest the cultures should get contaminated. With a sterile capillary pipette about 0.2 c.cm. of the thick emulsion is introduced in each container which is immediately attached to the rubber tube after duly flaming the mouth of the ampoule and the tip of the rubber tubing. Any number of containers can be attached in this way. It is the general practice here not to use more than 24 tubes at one sitting. It becomes cumbersome to use more tubes and also the chances of leakage of air into the system become greater. The degassing self-freezing procedure is employed. Preliminary degassing is accomplished by reducing the pressure slowly and allowing the system to remain under this low pressure for about half an hour. Further reduction of pressure is obtained up to the point where frothing just begins to occur. This accelerates the degassing process. Consequent on the rapid evaporation taking place from the material the temperature of the substance falls and freezing sets in with a suddenness very much like the sudden crystallization of supersaturated solutions. In this manner, the advantages of rapid freezing are obtained without the use of refrigerants, such as dry ice bath with alcohol, ether, acetone, etc. When degassing is complete and the material is frozen, the pump is allowed to evacuate to the limit of its capacity. By the time the frozen material has reached the fusion temperature, the air pressure is reduced to such an extent that moisture is taken up by the drierite with sufficient rapidity to maintain the material in the frozen state. Drying from the

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tests are concerned) were obtained with solutions that had been filtered through an asbestos filter. These results are recorded to draw attention to the importance of control by sterility tests of injectable substances.