## A SIMPLE METHOD FOR FILLING AMPOULES.

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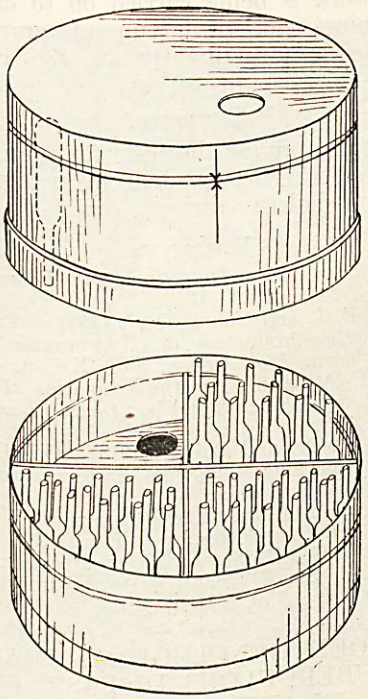
Wright (1912) described a method for filling ampoules based on the principle of first exhausting the air from the ampoules and then filling by atmospheric pressure. Since then many elaborate pieces of apparatus have been devised based on the same principle, but these are all expensive and not readily obtainable. The modification described below has the advantage that the apparatus required may be found in every laboratory, and the ampoule containers are of such a nature that they can readily be obtained in the market and cost only a few annas each. Thus, a number of ampoules can be filled without the necessity of buying large and expensive apparatus.

### The Technique.

*Ampoule containers.*—These are small aluminium boxes of a kind ordinarily utilised for the storage of food, and are readily obtainable in any market. A box (3 inches by 2



inches) holds about forty one-cubic-centimetre ampoules and a slightly larger one ($4\frac{1}{2}$ inches by 3 inches) holds sixty two-cubic-centimetre ampoules, the cost is about 3 and 6 annas, respectively. One with a deep flat lid is selected, and an extra lid obtained to fit the bottom of the box. A somewhat better box with a screw-on lid can be obtained at a slightly higher price. A small hole about one centimetre in diameter is punched through both the bottom of the box and the extra cover which is placed over the bottom. This serves to act as a valve-like opening to the ampoule container. A mark on the side of the box to indicate when the two holes are in apposition helps in the filling. A small sheet of aluminium is arranged inside the box so as to separate off the area in which the hole has been punched out. In the larger boxes, the box is divided by aluminium partitions into smaller compartments for the easy packing and subsequent handling of ampoules (*see* illustration). The ampoules are packed with their mouths pointing towards the lid, and the boxes sterilised.

The requisite amount of the material desired to fill the ampoules is poured by means of a sterile pipette into the ampoule container through the hole in the bottom of the box. The box is now placed with the lid downwards in any exhausting apparatus available—a Bulloch's apparatus is very satisfactory—and the air exhausted. When exhaustion is complete, filtered air is let in and the atmospheric pressure fills the ampoules. The box is turned upside down, so that the mouths of the ampoules are now pointing upwards, and the air again exhausted. In this way the fluid in the necks of the ampoules is emptied, rendering the sealing of the ampoules easy. A number of such boxes may be exhausted for filling at the same time. A hundred ampoules may be filled and sealed by one worker in half an hour and the risk of contamination is negligible.

This form of ampoule container has the disadvantage that larger ampoules than two cubic centimetres cannot be conveniently filled, but serves very well for the commonly used ampoules of one and two cubic centimetres.

*Summary.*

A cheap and readily obtainable ampoule container is described by the use of which a large number of ampoules can be filled in any laboratory that is equipped with an exhausting pump.

Reference.

Wright, A. E. (1912). *Handbook of the Technique of the Teat and Capillary Glass Tube.*

## A NOTE ON THE PRODUCTION OF A CYANOGEN RADICAL IN PEPTONE-WATER CULTURES OF CHOLERA VIBRIO.

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During the course of some investigations on cholera toxin it was noted that when a solution of ferric chloride was added to a peptone-water

culture of cholera a definite blood-red coloration was obtained. This led us to suspect the presence of a thiocyanate in the culture and further examination was undertaken.

The peptone water used was a 1 per cent. solution of Difco Bacto-peptone with 0.5 per cent. sodium chloride and a hydrogen-ion concentration of pH 8. Flasks containing 500 cubic centimetres of peptone water were inoculated with cholera culture and incubated at 37° for 72 hours. The culture was then acidified with tartaric acid and distilled. The distillate was received into a weak solution of caustic potash and the following tests as described by Autenrieth (1928) applied to the distillate.

I. Thiocyanate test. This test was found to be definitely positive.

II. Silver-nitrate test. A cheesy precipitate of silver cyanide, soluble in ammonia and insoluble in dilute nitric acid, was obtained.

III. Prussian-blue test. This test gave a bluish-green coloration which on standing for a few hours deposited traces of typical blue on the side of the tube. Although a frank Prussian-blue test for the presence of the HCN was not obtained the reaction is highly suggestive of the presence of small quantities of HCN (Vorländer, 1913).

IV. Vortmann's nitro-prusside test. (Vortmann, 1886). This test gave a greenish-yellow coloration showing the presence of minute traces of hydrocyanic acid (HCN).

The above tests performed with the distillate of uninoculated peptone water were negative in all instances.

A constant stream of filtered air was bubbled through a peptone-water culture of cholera vibrio acidified with tartaric acid and gently heated, and then passed through a weak solution of caustic potash; after four hours the tests were applied to the caustic potash solution. All the tests gave positive results, whereas control experiments—using uninoculated peptone water—were negative.

The stools from a severe case of cholera were distilled, and gave a positive silver-nitrate test.

The above experiments were repeated using 60 strains of cholera vibrio; with the majority of them the tests were positive, though some very old laboratory cultures failed to give the tests.

*Cholera-like vibrios.*—The following strains were grown in peptone water for 72 hours; the cultures were then tested for the presence of the cyanogen radical.

Peptone-water cultures of *B. dysenteriæ* (Shiga) and *B. paradysenteriæ* (Flexner), *B. typhosus* and *B. coli* gave negative results.

The experimental evidence is sufficient to conclude that some cyanogen radical is formed in peptone-water cultures of cholera vibrio. It may be hydrocyanic acid and in this connection it is interesting to note that Emerson, Cady and Baily (1913), and Clawson and Young (1913) reported the formation of hydrocyanic acid (HCN) by *Pseudomonas pyocyanea* in acid peptone water. The reactions may be due to some organo-cyanogen compound derived either from dehydration of amides or from some amino-acid by decarboxylation followed by oxidation (Dakin, 1916). What substances or which amino-acid is responsible for the production of the cyanogen radical in cultures of cholera vibrio is as yet unknown, and whether these compounds play any part in the symptomatology of cholera is yet to be determined. Further work is being carried on to determine which amino-acid, or group of amino-acids, yields the largest quantity of the cyanogen radical.

| Strain. | Source. | The CN test. |
|---|---|---|
| Vibrio 153 | Water | Negative. |
| Vibrio 216 | „ | „ |
| Vibrio 417 | „ | Strongly positive. |
| Vibrio 431 | „ | „ |
| Vibrio 467 | „ | Negative. |
| Vibrio 479 | „ | „ |
| Vibrio 534 | „ | Strongly positive. |
| Vibrio 309 | Man | Negative. |
| Vibrio 633 | Clinical cholera | „ |

*Summary.*

A substance giving the tests for the cyanogen radical has been detected in peptone-water cultures of cholera vibrio.

References.

Autenrieth (1928). *Detection of Poisons.* Sixth Edition, pp. 26, and 29—31.

Clawson, B. J., and Young, C. C. (1913). Preliminary Report on the Production of Hydrocyanic Acid by Bacteria. *Journ. Biol. Chem.*, Vol. XV, p. 419.

Dakin, H. D. (1916). On the Formation of Cyanides by Oxidation of Amino-acids. *Journ. Biol. Chem.*, Vol. X, p. 319.

Emerson, Cady, and Bailey (1913). On the Formation of Hydrocyanic Acid from Proteins. *Journ. Biol. Chem.*, Vol. XV, p. 415.

Vorländer, D. (1913). The Prussian Blue Reaction. *Berichte Deut. Chem. Ges.*, Vol. I, p. 181.

Vortmann, G. (1886). A New Reaction for the Detection of Small Quantities of Hydrocyanic Acid. *Monatsh. Chem.*, Vol. VII, p. 416.

## THE ROENTGENOLOGICAL DIAGNOSIS OF TUBERCULOUS DISEASE OF THE LUNG, IN ADULTS AND CHILDREN.

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Great strides have been made in the last few years in the radiology of the chest. Many