LETTERS TO THE EDITOR

The Estimation of *d*-Tubocurarine Chloride

SIR,—A method for the estimation of *d*-tubocurarine chloride, suitable for standardisation of injections, depending on the development of a blue colour with Folin-Ciocalteu phenol reagent, has been described by Foster[1], and its application in the assay of the total quaternary alkaloids in crude curare has been described by Foster and Turner[2]. In our hands this method has not been found to give results more accurate than the ±5 per cent. claimed, and, as stated, it is certainly necessary in all cases to prepare a standard at the time the assay is made. The issue of the Therapeutic Substances Amendment Regulations, 1948, has emphasised the necessity for accurate control of this preparation. We have found the qualitative colour test given in these regulations—the development of a cherry-red colour when a few crystals of the substance are added to 0·5 ml. of Millon's reagent—to form the basis of a suitable colorimetric assay, accurate to ±2 per cent. and requiring less manipulation than with the Folin-Ciocalteu reagent.

To prepare Millon's reagent pure mercury is dissolved in twice its weight of nitric acid B.P., heating if necessary to complete solution. The solution is diluted with twice its volume of water, allowed to stand overnight and filtered if necessary. Standard colour solutions are prepared as follows. A standard 0·1 per cent. solution of *d*-tubocurarine chloride in water is prepared. 1, 2, 3, 4 and 5 ml. portions of this solution are diluted to 10 ml. with water and 5 ml. of Millon's reagent is added to each. A blank is prepared from 10 ml. of water and 5 ml. of reagent. The contents of each tube are thoroughly mixed and allowed to stand at room temperature for 2 hours. (Unless a standard solution is set up side by side on each occasion a test is made, it is desirable to standardise a temperature, e.g., 20 ± 1° C., at which colour development is allowed to proceed.) Maximum colour development is attained at this time, the variation in colour for a few minutes on either side being negigible. Shortly after 2 hours precipitation commences in the more concentrated solutions. Light transmission is measured on a suitable photoelectric absorptiometer, using a cell having a light path of 2 cm. and a filter with a maximum transmission at about 430 mμ. A straight line graph is obtained by plotting the logarithmic (density) readings against concentration. For the assay of a sample 2 mg. is a convenient quantity to use and a volume containing this amount is diluted to 10 ml. with water and 5 ml. of reagent added. After standing at room temperature for 2 hours, the colour is compared against a blank, which should contain in similar proportions any additional solvent known to be present in the test sample.

Ethyl alcohol, benzyl alcohol, glycerol and sodium metabisulphite, any of which may be encountered in injections, do not interfere with the development of the colour, and it is unlikely that any inorganic salts which may be used as buffers, or to prepare isotonic solutions, will affect the results. Phenol and chlorocresol interfere, producing a pink colour similar to that given by the alkaloid, and may be removed prior to colour development by extraction with chloroform, as described by Foster.[1]

Duncan, Flockhart and Co., Ltd., Edinburgh. A. M. PRYDE,
January 19, 1949. F. R. SMITH.

REFERENCES

1. Foster, *Analyst*, 1947, **72**, 62.
2. Foster and Turner, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 228.