556 NOTES

from the other five extracts were pooled in an identical manner preparatory to colorimetric protein determination⁵. Pooling tubes according to a standard profile thus allows comparison of protein distribution between corresponding peaks from one set of chromatographic analyses to another.

The apparatus described provides a system whereby up to six protein samples can be eluted simultaneously from chromatographic columns under identical conditions by the automatic introduction of a succession of solvents. The routine inclusion of a standard protein sample provides a check on the reproducibility of the analysis, thus allowing comparison between sets of chromatographic analyses.

C.S.I.R.O. Wheat Research Unit, North Ryde, N.S.W. (Australia) C. W. WRIGLEY

- D. H. SIMMONDS AND D. J. WINZOR, Australian J. Biol. Sci., 14 (1961) 690.
 N. G. ANDERSON, H. E. BOND AND R. E. CANNING, Anal. Biochem., 3 (1962) 472.
 A. G. M. MARR AND C. N. GILBO, Nature, 185 (1960) 853.

⁴ C. W. WRIGLEY, J. Sci. Food Agr., 14 (1963) 120.

O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR AND R. J. RANDALL, J. Biol. Chem., 193 (1951)

Received December 24th, 1962

J. Chromatog., 11 (1963) 552-556

Some remarks on the paper "Centrifugally Accelerated Chromatography of Steroids"

In a recent paper by Matthews and Cervantes¹ on centrifugal chromatographic separation of steroids, velocities of 200-250 r.p.m. were used and it was found that the steroids did not move from the line of application. These authors thought that "this is probably because at high velocities the solvent travels too fast for partitioning to occur". However, the present authors consider that the unsatisfactory results of separation were due to an unsuitable arrangement of the centrifugal chromatography, for the following reasons.

- (1) The velocity of 200-250 r.p.m. is not sufficiently high to cause a substantial acceleration of the mobile phase flow. According to our own experience the run will take about 30 min instead of 40, which is the necessary time for developing a standard circular chromatogram.
- (2) The velocity necessary to establish the partition equilibrium is much higher than the velocity of the solvent flow. Even a velocity of the mobile phase which amounts to ca. 1,700 r.p.m. will not interfere with the partition.

No reason can be seen why these compounds (hydrocortisone, cortisone and IIdesoxy-17-hydroxycorticosterone) should not be separated by centrifugal chromatography under high velocities and actual separations are shown in Fig. 1. These were performed at 300, 600, 900 and 1,200 r.p.m. Paper preparation and sample application were the same as described by Matthews and Cervantes, but Whatman paper No. 3