This procedure was now applied to the photchemical reaction of dehydroisoandrosterone. The amounts of the unchanged starting material and the resulting product were determined and plotted graphically against the irradiation time. As illustrated in Fig. 2a, transformation into the 13α-compound reached equilibrium in 150 min; on prolonged irradiation increasing amounts of an undesirable by-product appeared, whose structure was not characterized. Furthermore the reverse reaction[6] was also explored in the same manner as described above. Upon irradiation with U.V. light 13α-dehydroisoandrosterone was transformed back again into its epimer, the amount of which finally reached a constant value almost equal to that in the reverse reaction (see Fig. 2b). The reversibility of the photochemical interconversion of 17-oxo-13β-steroid and its 13α-epimer has been clarified in a quantitative respect.

The present method for direct quantitation of the irradiation product itself is very useful for establishing the optimal conditions for the synthesis of 13α-steroid, because of its reliability and simplicity.

Further studies on the gas chromatographic separation of C-13-epimeric androstanes having various substituents in ring D are being conducted in this laboratory and will be reported in the near future.

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Dry filling of capillary columns for liquid chromatography

We have previously described capillary teflon columns for adsorption and partition chromatography[1] and given funnel and pump filling liquid techniques for this type of column.

Working with the capillary columns we have found that a simpler dry filling technique offering many advantages in capillary column chromatography is just as effective for some column materials.

Dry filling technique

Manual filling. The simplest technique is to mount a funnel through a reducing swagelock fitting on top of a capillary teflon column as described earlier for liquid filling[1] of capillary columns. The column material is poured into the funnel and the column is then filled by tapping on the outside of the column and the funnel either using a finger or a vibrating tool.

Machine filling. If many columns are to be filled at one time, a machine filling technique is to be preferred. Fig. 1 shows the arrangement for this. A funnel holder is attached to a rotating shaker (A. H. Thomas, Philadelphia, Pa. 3623) and the shaker

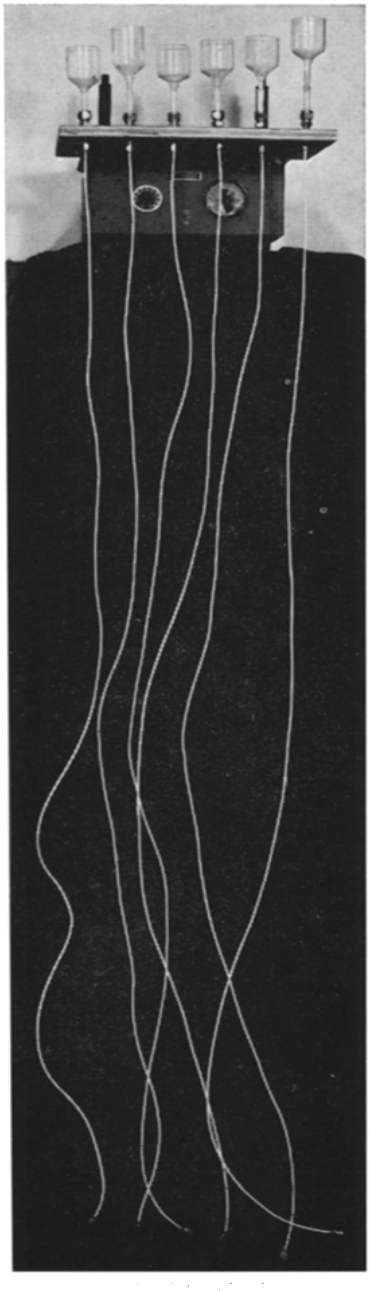

Fig. 1. Machine filling of capillary teflon columns. The filling funnels are attached to a shaking machine located 6 ft. above floor level. The capillary columns are screwed to the funnels through a swagelock fitting. The columns are capped at the bottom with a stainless steel cap.

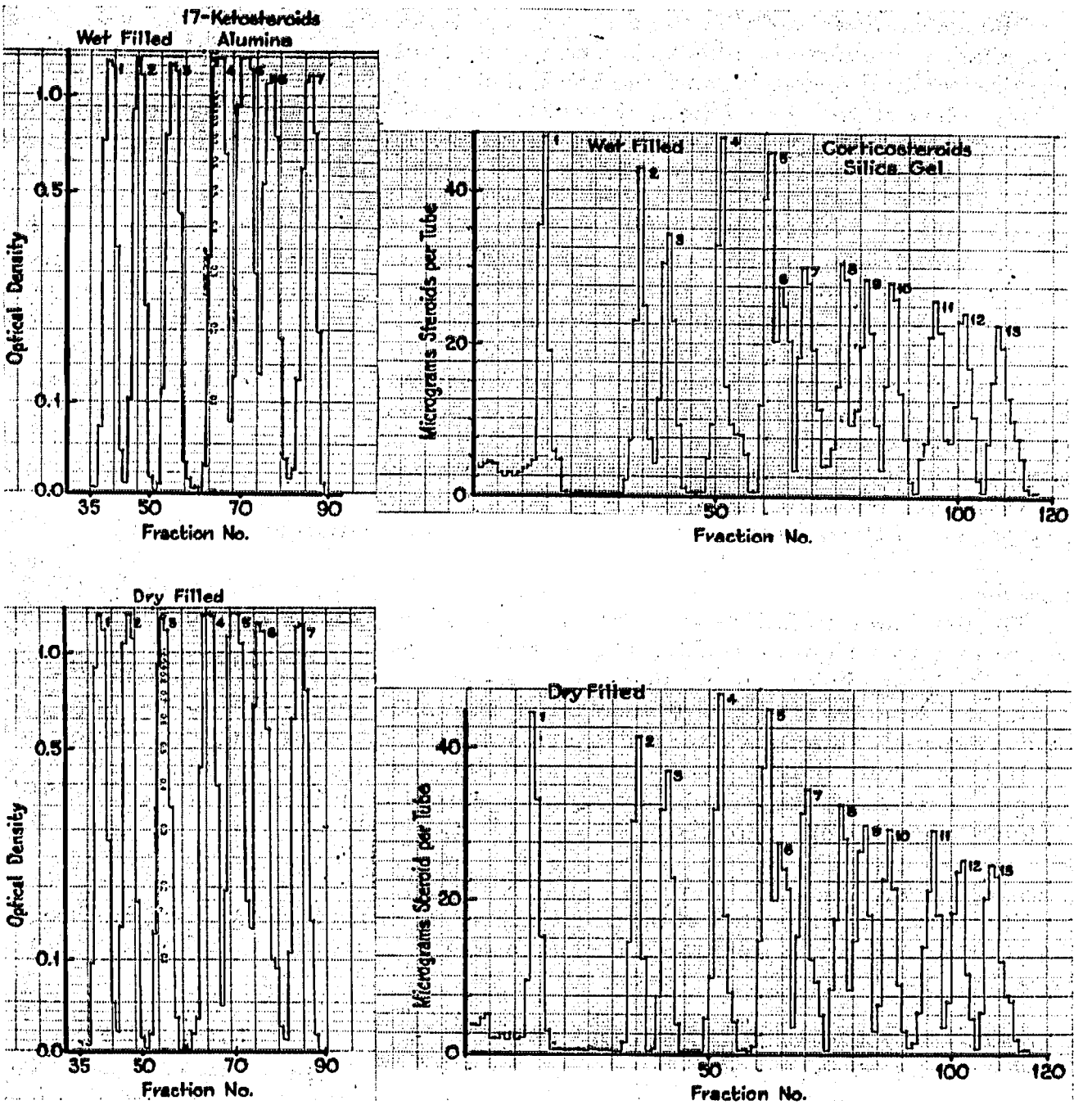

Fig. 2. A comparison of the relative efficiency of wet and dry filling techniques. To the left are chromatograms from the same dual column run using a wet filled alumina column (top) and a dry filled column (bottom) to separate seven common urinary 17-ketosteroids: 1 = dehydroisoandrosterone; 2 = androsterone; 3 = etiocholanolone; 4 = 11-ketoandrosterone; 5 = 11-ketoetiocholanolone; 6 = 11-hydroxyandrosterone; 7 = 11-hydroxyetiocholanolone. To the right are similar results from a comparison between wet and dry filled silica gel columns used to separate thirteen corticosteroids; 1 = desoxycorticosterone; 2 = dehydrocorticosterone; 3 = compound S; 4 = corticosterone; 5 = cortisone; 6 = dihydrocortisone; 7 = tetrahydro compound S; 8 = dihydrocortisol; 9 = cortisol; 10 = *allo*-tetrahydrocortisone; 11 = tetrahydrocortisone; 12 = *allo*-tetrahydrocortisol; 13 = tetrahydrocortisol. 6 ft. capillary teflon columns were used in both systems. The ketosteroid chromatograms were recorded in our automatic read-out system[2]. The corticosteroids were measured after overnight reaction with the Porter–Silber reagent.

placed on a stand about 6 ft. above the floor. The funnels are placed in the holder and the capillary columns to be filled attached to the funnels as shown in the illustration. The proper amount of column material is weighed out and poured into the funnels. The capillary columns are capped off at the bottom after having been plugged with glass wool. The shaker is now turned on and the columns will fill up in from 5–20 min depending on the column material used.

Performance

A comparison has been made between the wet filling technique described earlier and the new dry filling technique. This was done for two types of material. Aluminum oxide (Woelm, Eschwege, Germany) for adsorption chromatography and silica gel (Woelm) for partition chromatography.

A set of 17-ketosteroid standards were separated in a two-column run using a wet filled and a dry filled alumina column under identical conditions with the technique described elsewhere[2]. A set of corticosteroid standards were separated in a 6 h run on wet and dry filled silica gel columns using a gradient of acetone in chloroform[3].

It can be seen from Fig. 2 that there is no difference in performance and separatory powers of the column material when the two techniques are compared.

Discussion

The dry filling technique is particularly useful when many columns of the same type are prepared simultaneously. The machine filling technique can be easily expanded. The funnel holder on the shaker shown can be built to accommodate twenty columns at a time by attaching funnels on all sides. The columns once dry filled are more conveniently stored for later use in a closed container than liquid filled columns.

It is probable that not all column materials are suitable for filling with this technique. The technique would, however, appear to be preferable to liquid filling techniques when it can be applied.

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