potassium D-3-phosphoglycerate, 50 (containing 0.12 μM D-2,3-diphosphoglycerate); $MgSO_4$, 10; tris(hydroxymethyl)aminomethane, pH 7.0, 100; and 1.2 enzyme units of mutase-free enolase*. Up to 0.25 enzyme units of mutase may be taken for assay. In the enolase assay DL-2-phosphoglycerate is substituted for the D-3-phosphoglycerate. Protein is estimated by a semimicro adaptation of a standard Biuret method.

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* Prepared from Anheuser-Busch dried bottom yeast strain BSC by a modified procedure of the method of WARBURG AND CHRISTIAN[3]. We are indebted to Dr. D. P. WALLACH of this laboratory for aid in this preparation.

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Preparation of paper for quantitative chromatography of corticosteroids

Quantitative chromatography of steroid mixtures is usually performed on columns[1,2,3] despite the existence of paper methods of high resolving power[4,5]; yet paper chromatography offers considerable advantages both in simplicity and reproducibility. The drawback to paper work has been the high blank value of the eluate with many methods of determination, including fluorimetry in sulphuric acid[6] and in potassium *tert*-butoxide[7], ultraviolet spectrophotometry and arsenomolybdate reduction[8]. Washing of papers in the Soxhlet apparatus has not been impressive in reducing blank values, but the following technique has been consistently satisfactory in over a year's use in this laboratory.

Whatman No. 2 "Paper for Chromatography" is cut into 50 × 1.5 cm strips. These are washed chromatographically for 24 hours with 2N ethanolic sodium hydroxide, prepared from freshly redistilled 95% ethanol. The papers are then washed with distilled water until the eluate is free from alkali, and finally with 95% ethanol for three hours. After drying they may be stored indefinitely.

SWEAT's sulphuric acid-induced fluorescence has been employed by us in plasma corticosteroid estimations involving paper chromatography. When 8 cm lengths of the prepared strips are eluted with absolute ethanol and compared fluorimetrically with 1 μg cortisol as standard, blank values never exceed 10% of the cortisol figure, and are usually in the range 3–6%. The eluate from unwashed paper gives a value 1–3 times greater than the standard.

In absorptiometry at 240 mμ, the paperblank reading is 5–10% of that obtained with 10 μg cortisol. Limited experience with the potassium *tert*-butoxide method[9] indicates that blank values are even lower than with sulphuric acid.

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