Some observations on the elution of progesterone from silica gel.

D.A. Stansfield.

(Department of Biochemistry, Queen's College, University of St. Andrews, Dundee)

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A previous communication (Stansfield and Cargill, 1963) described a quantitative method for the extraction of progesterone by means of two dimensional thin layer chromatography on silica gel. The recoveries reported in the above paper were of the order of 62%.

Recently, however, recoveries of a much lower level have been experienced (approx. 30-35%) although the linearity of the progesterone extractions was maintained in all cases.

The cause of these low recoveries has been shown to be inefficient elution of progesterone from the silica gel plates after completion of the chromatographic steps. Samples of the eluant used, methylene chloride, appear to have a variable eluting power, the variability being due, in part at least, to the moisture content of this solvent.

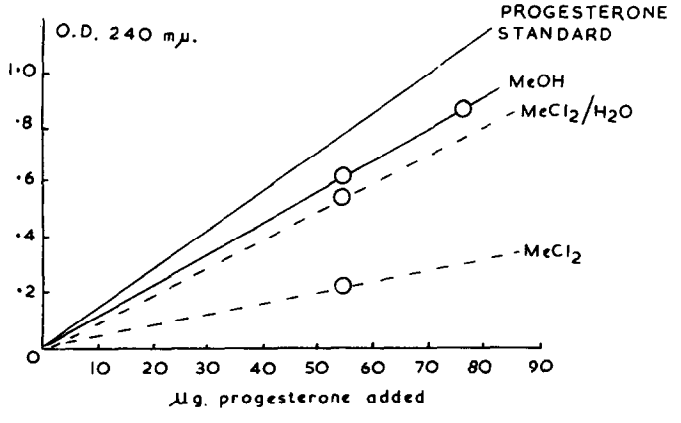

Fig. 1. Elution of progesterone from silica gel after chromatography of lipid extract of bovine corpus luteum homogenate, supplemented with the indicated amounts of progesterone.

It is apparent from Fig. 1 that a sample of methylene chloride which is a poor eluting agent is vastly improved in this respect by saturating it with water. Fig. 1 also shows that methanol is a more efficient eluting agent than wetted methylene chloride, and it is suggested that this solvent should be used routinely in the final step of the method of Stansfield and Cargill. The reasons for the increased eluting power of water saturated methylene chloride are not at present clear, but the following suggestions are made:

a) The water may serve to hydrate the silica gel to such an extent that a partition system may be set up between the "aqueous" silica gel and the less polar methylene chloride and this may be more conducive to removal of the progesterone than a straightforward desorption effect.

b) The silica gel appears to disperse into a finely particulate form more readily in methanol and the water saturated methylene chloride, than in the "dry" methylene chloride thus allowing a better penetration of the particles by the solvent.

References

Stansfield, D.A., and Cargill, D.I., Biochem. Biophys. Res. Comm. 13,231 (1963)