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The role of elution solvent in quenching of ITTRICH estrogen fluorescence reaction observed with silica gel eluates

It was originally observed by ITTRICH[1] that "Kober chromogens" formed in the reaction of estrogens with sulfuric acid and hydroquinone are extractable in a 2% solution of *p*-nitrophenol in chloroform or tetrabromomethane. Fluorimetric measurement on this extract provides a very sensitive procedure for estrogen analysis and has been used by several workers[2–5], for measuring microquantities of estrogens in biological fluids. However, this reaction is subject to quenching due to a variety of factors and special care has to be taken in applying it to paper or silica gel thin-layer eluates. Thus, HOBKIRK[6] observed that while he recovered about 95% of the radioactivity after chromatographing pure labeled estrogens on silica gel thin-layer plates, application of the ITTRICH fluorescence reaction to the eluates gave no fluorescence at all. During the course of our work on estimating estrogens from bovine ovarian perfusates we also encountered considerable quenching of the fluorescence reaction when applied to silica gel eluates. Therefore, we studied the factors involved in the quenching effect of silica gel eluates. The experiments showed that the degree of quenching is dependent on the kind of solvent used for elution of an estrogen from silica gel.

Since the information obtained may be useful to workers in this field, we wish to report it here.

(4-^{14}C)-Estradiol-17β (New England Nuclear Corp.) was chromatographed on 3 mm Whatman paper in the system hexane–toluene–methanol–water (66:33:85:15). Radioactive material running parallel to standard estradiol-17β was eluted and used for further experiments. It was mixed with recrystallized non-radioactive estradiol and a working solution (specific activity 28530 d.p.m./μg) was prepared in ethanol.

Silica gel (Merck) thin-layer plates (20 × 20 cm) were prepared in the usual way. Known aliquots (1–1.5 μg) of the working estradiol solution were spotted on a plate (three samples per plate) along with standard estradiol in parallel lanes and the plates were developed in methylene chloride–methanol (96:4). The following procedure was then followed for each sample: The area running parallel to the standard estradiol was scraped off into a tube and eluted by shaking the silica gel with a solvent (4 × 2 ml) on a Vortex mixer. The pooled solvent was evaporated under nitrogen at 40° and the residue dissolved in 1 ml of ethanol. Appropriate aliquots were then taken for the determination of mass by the ITTRICH fluorescence reaction as described by MAHESH[7], and for counting of radioactivity on a Tri-Carb liquid scintillation counter (the efficiency for ^{14}C was 75%). The difference between percent recoveries calculated on the basis of estimated mass and that obtained by counting of recovered radioactivity (the latter was always the greater of the two) gave the degree of quenching in each case. The quenching was expressed as

$$Q = \frac{\text{recovery on basis of fluorescence reaction}}{\text{recovery on basis of radioactivity}}$$

In an ideal situation where no quenching is observed, the ratio Q should be equal to unity.

Methanol, ethyl acetate, chloroform (Mallinckrodt, all redistilled) and ether (Mallinckrodt) were used for elution in different experiments. The quenching observed with the respective eluates is shown in Table I.

TABLE I

QUENCHING OF ITTRICH FLUORESCENCE REACTION OBSERVED WITH ESTRADIOL SAMPLES ELUTED FROM THIN-LAYER SILICA GEL. EFFECT OF ELUTION SOLVENT

Elution solvent	% *recovery of spotted* (4-^{14}C)-*estradiol*[a]	*Quenching index*[b]
Methanol (14)	89.9 ± 3.8	0.166 ± 0.027
Ethyl acetate (14)	85.0 ± 6.2	0.645 ± 0.060
Chloroform (14)	86.3 ± 6.9	0.919 ± 0.035
Ether (14)	92.1 ± 4.6	0.913 ± 0.021

[a] On basis of radioactivity.
[b] Q = % recovery on basis of fluorescence reaction/% recovery on basis of radioactivity.

It will be seen from Table I that methanol and ethyl acetate elution caused considerable quenching while chloroform and ether eluates produced only marginal quenching. The quenching effect of methanol and ethyl acetate eluates could be traced to the solvents themselves and just evaporating estradiol samples with these solvents caused almost the same degree of quenching. Extensive purification of these solvents may do away with this effect. However, since solvents like ether and chloroform can be used without any treatment they are to be preferred for eluting estrogens from silica gel if the eluates are to be used for fluorescence reaction.

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