Correspondence and Short Communications

Comments on published articles, short communications of a preliminary nature, case reports, technical notes and the like are accepted under this heading. The articles should be short and concise and contain a minimum of figures, tables and references.

UNBOUND, BIOLOGICALLY ACTIVE OESTRADIOL, SEX HORMONE-BINDING GLOBULIN, EPIDERMAL GROWTH FACTOR AND ELECTROLYTES IN BREAST CYST FLUID

Several studies have shown that women with gross cystic breast disease (cysts of more than 3 mm in diameter), a common condition affecting about 7% of women in the western hemisphere, may be at increased risk of developing breast cancer (1, 2). Since both oestradiol (E_2) and epidermal growth factor (EGF) may play a role in the development of breast cancer (3, 4), we thought it pertinent to measure the concentrations of unbound, biologically active E_2 and EGF in breast cyst fluid. In addition, we studied the relationship between intracystic concentrations of E_2 and sex hormone-binding globulin (SHBG) as this has not been reported in the literature.

Material and Methods. Breast cyst fluid was obtained by needle aspiration of 25 breast cysts from 25 women (aged 30 to 60 years with a median age of 45 years) who attended the Breast Clinic at St. Mary's Hospital, London. The samples were centrifuged and the supernatant stored at −20°C until analysis.

Anti-E_2 antiserum raised against oestradiol-6(0-carboxymethyl)-oxime bovine serum albumin in goats was a gift from Dr. G. Knaggs. Anti-human EGF antiserum and pure urogastrone (EGF isolated from human urine) was purchased from Amersham International plc, Amersham, Bucks., UK. The SHBG immunoradiometric kit was obtained from Farmos Diagnostica Ltd., Organon Teknika Ltd., Cambridge, UK. The Dianorm equilibrium dialysis system was obtained from MSE Scientific Instruments, Crawley, UK.

Total E_2 was estimated with a method previously described (5). The sensitivity of the assay was 18 pmol/l and the intra-assay and interassay coefficients of variation were 6.5% and 10.7% respectively.

The percentage of unbound E_2 in breast cyst fluid was measured using a dianorm equilibrium dialysis machine. This methodology has been described earlier (6). The principle of the technique is to dialyse breast cyst fluid against (2, 4, 6, 7-^{3}H)E_2 (approximately 10 000 cpm) until equilibrium is reached. The unbound E_2 concentration was calculated by multiplying the percentage of unbound E_2 by the total E_2 concentration. The intra-assay and interassay coefficients of variation were 3.7% and 12.9% respectively.

SHBG was measured by an immunoradiometric method (Farmos Diagnostica kit). Breast cyst fluid was assayed in 2 dilutions, 1 in 5 and 1 in 10. The sensitivity of the assay was 0.5 nmol/l. The intra-assay and interassay coefficients of variation were 2.1% and 6.5% respectively.

EGF was measured by a double antibody radioimmunoassay. Sample/standard (0.1 ml) was incubated with 0.1 ml of label (about 8 000 cpm), 0.1 ml of antiserum (working dilution of 1 in 30 000) and 0.2 ml of assay buffer (0.05 mol/l phosphate buffer, pH 7.4, containing 0.2% bovine serum albumin) at 4°C for 3 days with a further day's incubation following the addition of donkey anti-rabbit second antibody.

The sensitivity of the assay was 0.04 ng/ml and the intra-assay and interassay coefficients of variation were less than 10%.

Sodium and potassium concentrations were measured using an indirect ion selective electrode (Beckman Electrolyte 2 Analyser).

The data were not normally distributed. Non-parametric statistics were, therefore, used. Correlations were assessed using Spearman's rank correlation method (r_s = Spearman's rank correlation coefficient). Results were considered to be statistically significant when $p < 0.05$.

Results. The concentration ranges and median concentrations of E_2 (total and unbound), SHBG, EGF, sodium and potassium are shown in the Table.

No correlation was found between the intracystic concentration of total E_2 and SHBG. A negative correlation ($r_s = -0.431$, $p < 0.05$) was obtained between the intracystic concentrations of unbound E_2 and SHBG (Figure). No correlations were found between intracystic concentrations of EGF and total or unbound E_2.

Discussion. The exact mechanism by which SHBG, a protein which is synthesised in the liver, enters breast cyst fluid from plasma remains an enigma. Entry into cyst fluid, however, appears to be better in cysts with high intracystic sodium to potassium ratios. A positive correlation was obtained between SHBG

Table

Concentration ranges and median values of analytes measured (n = 25)

Analyte	Range	Median
Total E_2	50–1 000 pmol/l	103 pmol/l
Unbound E_2	4.3–119 pmol/l	9.7 pmol/l
SHBG	1.3–143 nmol/l	3.5 nmol/l
EGF	1.8–570 ng/ml	178 ng/ml
Sodium	27–145 mmol/l	128 mmol/l
Potassium	4–124 mmol/l	19 mmol/l

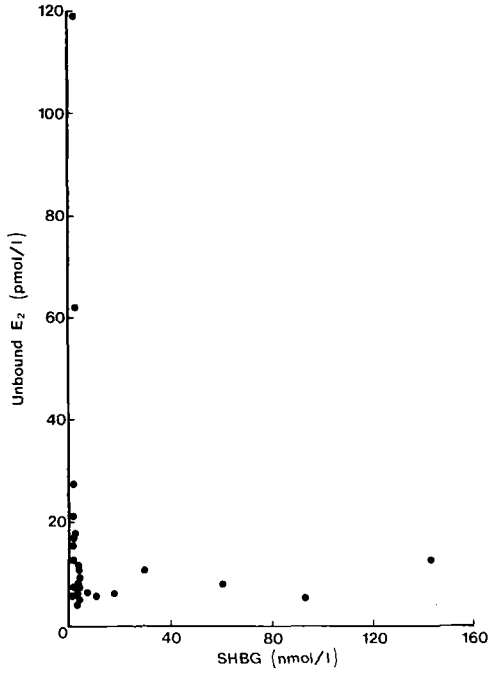

Figure. Concentrations of unbound E_2 versus SHBG ($r_s = -0.431$, $p < 0.05$, $n = 25$).

concentrations and sodium to potassium ratios in breast cyst fluid ($r_s = 0.453$, $p < 0.02$). Intracystic sodium to potassium ratios may reflect the nature of the epithelial lining of breast cysts, ratios of less than three may indicate the presence of apocrine epithelial lining while ratios of greater than three may indicate the presence of attenuated epithelial lining (7).

Concentrations of testosterone and dihydrotestosterone have been shown to be higher in breast cyst fluid than total oestradiol (8). In cysts with low intracystic sodium to potassium ratios, where SHBG concentrations are lower, the presence of high concentrations of testosterone and dihydrotestosterone, both of which bind more strongly to SHBG, may be the explanation for the higher proportion of unbound E_2 present in these cysts.

E_2 in breast cyst fluid may be derived by metabolism of dehydroepiandrosterone sulphate by the cyst wall (9). EGF was present in high concentrations in breast cyst fluid, supporting the findings of Jaspar & Franchimont (10). Concentrations of EGF are almost undetectable in plasma (11). It seems likely that EGF in breast cyst fluid may arise from local synthesis and secretion of EGF by mammary epithelial cells rather than the active transport of EGF from plasma into cyst fluid. Wide-ranging concentrations of total and unbound E_2 and EGF were found in breast cyst fluid. The significance of this finding is unknown.

In view of the possible role which both unbound, biologically active E_2 and EGF may have in carcinogenesis, we postulate that women who have high intracystic concentrations of both unbound E_2 and EGF may be at higher risk of breast cancer. Longitudinal studies on women with gross cystic breast disease assessing the predictive value of intracystic concentrations of E_2 and EGF should perhaps be carried out.

Key words: Breast, cyst fluid, oestradiol, sex hormone-binding globulin, epidermal growth factor.

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