7th International Congress of Clinical Chemistry
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Hormones, Lipids and Miscellaneous

Editors: J.-P. FELBER, Lausanne, and J.-J. SCHEIDEGGER, Geneva

With 235 figures and 71 tables

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Preface

This volume gathers a number of papers relating to the clinical chemistry of hormones. Readers interested in peptide hormones will particularly appreciate the chapters on radioimmunoassays which include contributions from some of the best specialists of this rapidly developing field.

The chapter on lipids contains studies made on humans under experimental conditions. The remainder of the book consists of contributions to the symposium on detection of metabolic inborn errors, and of a number of papers assembled under the title "miscellaneous", some of which have the merit of dealing with unusual aspects of clinical chemistry.

We thank Professor J. P. Felber who organized and edited the symposia on radioimmunoassays in such an excellent manner.

M. Roth
General Editor

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7th int. Congr. clin. Chem., Geneva/Evian 1969; vol. 3: Hormones, Lipids and Miscellaneous, pp. 1-5 (Karger, Basel/München/Paris/New York 1970)

Endocrine Abnormalities in Human Breast Cancer

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Many investigations have been conducted to determine what factors are associated with breast cancer. These have included socioeconomic factors, familial aggregation, endocrine function, and more recently, urinary steroid excretion patterns. No previous study has attempted to coordinate these various factors in an integrated way.

The present report describes a study directed to families in which two or more members have already developed breast cancer and where the family members are being subjected to clinical, radiographic, pathologic, and endocrine evaluations. This combined approach has high promise for identifying family members with early lesions of breast cancer or possibly those who may be at high risk for the subsequent development of the disease. The resulting data should also have utility in evaluating the genetic aspects of breast cancer and, specifically, whether the disease is comprised of one or more subtypes, which might be distinguishable by genetic, pathologic, radiologic, and/or endocrine criteria.

Only preliminary results are presently available. These pertain to clinical and pathologic characteristics of 399 patients with family histories of breast cancer among a total of 5,775 such cancer patients on file at The University of Texas M. D., Anderson Hospital and Tumor Institute at Houston, for the period from 1944–1967.

In a comparison of these two groups of patients with and without family histories of breast cancer, ANDERSON [1968] found differences with regard to: (1) age of first admission (the closest approximation to the age at onset); (2) tumor classification; (3) two different histories of obesity, hypertension, and diabetes within the familial group itself; and (4) lesion multiplicity. Familial patients with breast cancer had a younger age distribution and an

earlier average age at first admission than did non-familial patients. A young age has also been found to characterize other genetic varieties of neoplasms, whose average ages at onset are markedly earlier than the same neoplasms occurring sporadically in the general population.

It was noted in this study that familial patients with a history of obesity, hypertension, and/or diabetes had a distinctly older age distribution than did the 221 familial patients without these findings. The age distribution in the obese, hypertensive, diabetic group corresponded with the post-menopausal period. The age distribution in the other group without these findings corresponded with the pre-menopausal or post-menopausal periods.

Familial patients were also found to have an excess number of medullary carcinomas and more intraductal and lobular carcinomas than non-familial patients.

Another difference was that the familial group had significantly more bilateral occurrences of breast cancer than the non-familial group, a finding in keeping with other genetic cancers, which are also characterized by multiple and bilateral lesions.

The findings thus indicate the interesting possibility of at least two hereditary varieties of breast cancer: an early type which may be associated with ovarian estrogens and/or androgen insufficiency; and an older group with an adrenocortical imbalance. The endocrine part of this study, therefore, is extremely important in evaluating this possibility.

For many years, the modification of the internal steroid environment by endocrine ablation and hormone administration has continued to be a mainstay in breast cancer therapy. Palliation consists of removing the ovaries, the adrenals, or the pituitary, either singly or in combination. Hormones are also discriminatingly administered. Steroids, and estrogens in particular, are known to influence the growth of normal and neoplastic mammary tissue.

In a study in England, BULBROOK and HAYWARD [1967] showed abnormal urinary excretion patterns of the steroid hormones in breast cancer patients. They expressed this variance as a weighted deviation of etiocholanolone and corticosteroids. Their data were determined prior to the onset of clinical signs of breast cancer. However, of 22 cases of breast cancer which were later detected in a prospective study of 5,000 women, 18 had abnormal steroid patterns prior to the development of breast cancer. Since then, three more breast cancers with abnormal steroid patterns have appeared [BULBROOK, 1968]. However, BULBROOK [1966] conceded that several other factors could

influence the predictions, including the interval between mastectomy and reappearance of disease, age, menopausal status, and nationality.

LEMON *et al.* [1966] believe that attempts to demonstrate differences in the ratio of non-carcinogenic estriol to carcinogenic estrone and estradiol between breast cancer cases and controls have been inconclusive. They feel that the discrepancy in results obtained by different investigators is the result of differences in the methods and the timing of patient selection for study. They themselves find a marked increase in the urinary quotient of estriol/estrone+estradiol among normal women as opposed to those with breast cancer. With repeated pregnancy and later post-menopausal ovarian atrophy, it is likely that the quotient would rise in subnormal excretors and thus reduce the likelihood of late cancer. They suggest that normalization of a precancerous metabolic imbalance between impeded and active estrogens would also contribute to arrested growth of estradiol-dependent metastases.

The endocrine part of the present investigation is an extension of BULBROOK's studies with modifications and the inclusion of other hormones. The urinary steroid excretion patterns of women belonging to families in which two or more cases of breast cancer have already occurred will be compared with controls. The controls consist of females from the general population of the Texas Medical Center and without a family history of breast cancer, but matched for age, marital status, parity, and menstrual history to those in the familial series. Thermograms and mammograms have already been completed on the controls and have just begun on the familial women. The latter will have, in addition, physical breast examinations.

For endocrine analyses, a 24-h urine sample is requested. The 17-ketosteroid determination is by the RUCHELMAN and COLE [1966] method, utilizing gas chromatography. This consists of mild hydrolysis with sulfuric acid, followed by extraction, sodium hydroxide wash, drying, and evaporation of solvent. Derivatives (trimethylsiyl ethers) are formed for gas chromatography, on a 12 ft$\times$4 mm glass column of 4% XE–60 phase, with automatic sample injection [RUCHELMAN, 1966]. A computer program is in the process of being written to cover these urinary determinations.

Corticosteroids are being analyzed by a modification of the PETERSON *et al.* [1955] method, with enzymatic hydrolysis in a buffered solution, dichloromethane extraction, freezing out of the acqueous phase, and colorimetry of a phenylhydrazine-reacting group.

A procedure for low-level estrogens is presently being developed in this laboratory, consequently, aliquots of urine are being frozen for future use.

This method will entail hydrolysis by a mixed glucuronidase-sulfatase enzyme preparation, extraction with ether, then sodium hydroxide. Thin-layer chromatography will remove interfering substances prior to whole-sample injection of the estrogens as free steroids on gas chromatography. The quantitation will be similar to that elaborated in the solubility studies of RUCHELMAN [1969] of the three major estrogens, where no derivatives were used for gas chromatography.

Future plans call for evolving a new method of analysis of hydroxycorticosteroids by gas chromatography, involving fractionation into individual components for discerning true patterns.

MEYER [1967] points out that the new technologic developments permit a rapid, accurate analysis of detectable steroid differences between patients. This gives assurance that future research will lead to predictions of response.

Among the hormone steroids, specific chemical anomalies may be best detected by a discriminant function [similar to that described by BULBROOK], or by a ratio, either of which could consist of a combination of androgens and hydroxycorticosteroids, of androgens and estrogens, or of estrone, estradiol, and estriol alone.

Possibly, also, some endogenous combination of hormones may produce a completely new type of steroid that may be present in the urine of these high-risk individuals. This could be present as a metabolite of the osteolytic steroid, the 'spreading factor' found in sera of a number of breast cancer patients by BROWN and MARRACK [1968].

As previously pointed out, gas chromatography is the method of choice for detecting abnormalities in the steroids. If specific steroid abnormalities are detected in genetically high-risk women, this could have ultimate bearing on breast cancer control. It might then be possible to correct this endocrine abnormality during the initial stages of disease development before the disease becomes irreversible.

Summary

This is a prospective-type study with a twofold purpose: (1) evaluation of endogenous hormonal steroids in concert with mammography and thermography in an attempt to detect and identify women with very early lesions of breast cancer; and (2) correlation of endocrine, histologic, and radiologic data with genetic data in order to clarify the role of genetic factors and to discriminate high-risk families.

Soon endocrine anomalies may be recognized early enough to have ultimate bearing on breast cancer control at the initial stages of the disease.