Advances in Steroid Analysis '84

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Szeged, Hungary, June 12–14, 1984

edited by

S. Görög

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Volume 23 Advances in Steroid Analysis '84. Proceedings of the 2nd Symposium on the Analysis of Steroids, Szeged, Hungary, June 12–14, 1984
edited by S. Görög

PREFACE

This volume comprises the Proceedings of the 2nd Symposium on the Analysis of Steroids, held in Szeged, Hungary, June 12-14, 1984. The first symposium took place in Eger, 1981, and the Proceedings were published by the same publishers (S. Görög (Ed.): *Advances in Steroid Analysis*, Akadémiai Kiadó, Budapest, and Elsevier, Amsterdam, 1982).

Like in the case of the first symposium, the general aim of this recent meeting was again to provide an international forum of publication for steroid analysts working in biochemical, clinical, pharmaceutical and industrial laboratories, to present an overview on the state-of-the-art and current methodology of steroid analysis. In accordance with our hopes and aims, all these fields were well represented by leading experts from 17 countries, covering in their contributions the analysis of all important groups of steroids: hormones and other steroid drugs, sterols, vitamins D, bile acids, cardiac glycosides, sapogenins.

As the program of the symposium was methodology-oriented, the main chapters of this volume have been compiled in a manner to cover primarily individual analytical techniques. Some papers, which are not methodology-oriented, are collected into a "General" chapter. This is followed by a short chapter dealing with protein-binding and receptor-binding studies. The most comprehensive chapter is concerned with immunological (RIA, EIA, etc.) methods. Gas chromatography (mainly in conjunction with mass spectrometry) and high-performance liquid chromatography are equally well represented. The volume is concluded by

a chapter containing miscellaneous methods, such as thin-layer densitometry, photometry, enzymatic assays, and CD spectroscopy.

I should like to thank the members of the Organizing Committee, Drs. I. Faredin, T. Fehér, A. Laukó, J. Morvay and G. Szepesi, for their cooperation in organizing the Symposium, creating thereby the basis for the publication of this book.

S. Görög

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RADIOIMMUNOASSAYS

AUTOMATION IN STEROID ANALYSIS

R. VIHKO, E. BERTOFT*, T. LÖVGREN*, O. MÄENTAUSTA**
and E. SOINI*

Department of Clinical Chemistry, University of Oulu,
SF-90220 Oulu, Finland
*Wallac Biochemical Laboratory, Turku, Finland
**Research Laboratory of Farmos Diagnostica, Oulunsalo,
Finland

INTRODUCTION

Automation in routine and research laboratories aims at increasing the productivity of laboratory personnel primarily by reducing manual work. An equally important aim is to improve the quality and availability of laboratory results. These two main goals, to increase productivity and to improve quality also exist in laboratories performing steroid analyses.

TRENDS IN STEROID ANALYSES

Research Laboratories

As summarized in these proceedings by Sjövall, there is and will remain a need for sophisticated steroid analyses employing gas liquid chromatography-mass spectrometry. The general trend, however, has been towards employing immunological techniques in steroid analyses. Research laboratories frequently use home-made antibodies and tritiated labels and often employ fractionation and purification procedures prior to immunoassays. This kind of approach, although by necessity often operating with relatively limited numbers of specimens to be analyzed, easily leads to multiple radioimmunoassays because the number of steroids measured may be high (Sippel et al. 1978, Vihko et al. 1978). It is therefore obvious that in research laboratories also, there is a need for work-simplifying procedures to improve assay capacity and speed. Batch-wise analytical approaches also lead to avoidance of certain pitfalls in analytical procedures, such as in pipetting, times of incubation prior to separation etc. Hence there is also a need for semiautomated procedures in research laboratories.

Routine Laboratories

Routine laboratories fall into different categories in relation to their use of steroid analyses. Although there are large units concentrating on steroid analyses, usually including assays of cortisol, estradiol, progesterone, testosterone, estriol, aldosterone, dehydroepiandrosterone and its sulfate, androstenedione, 17α-hydroxyprogesterone and estrone, these assays often are only a part of workload of the radioimmunoassay or endocrine laboratory. Steroid analyses are often performed by personnel also engaged in analyses of other hormones such as protein and peptide hormones, several analytes relating to cancer detection, monitoring of fetal

well-being, anemia diagnostics and liver function. For example, our experience of steroid analyses in routine use stems from a laboratory performing approximately 1 million chemical analyses each year, of which approximately 100 000 are based on the use of immunological techniques. In such an environment, automation of steroid analyses must be compatible with the automation of a large number of other immunological techniques. At present, routine laboratory analyses of 41 different analytes are carried out using radioimmunoassay and 6 analytes are measured by using alternative labels in the assay. Hence, our approach to automate radioimmunoassays, including steroid analyses, demands several radioimmunoassays to be performed concurrently with no carryover between samples or between assays. The approach is a semiautomated batch-assay system. This is also compatible with our wish to use the same instrumentation in our research.

In conventional routine laboratories, the number of analyses of individual steroids does not rise to such high figures as seen in association with thyroid diagnostics, pregnancy monitoring, and analyses performed in association with diabetes diagnostics. However, there are certain trends which suggest that the situation is changing. The expected advent of analyses of salivary steroids (Riad-Fahmy et al. 1982) aims at more frequent sampling in individual patients to strengthen the diagnosis of a number of clinical problems such as infertility, monitoring of pregnancy and follow-up of treatment of congenital adrenal hyperplasia, to mention a few. There are also special applications of steroid analyses, such as optimizing insemination in cows, which involve large numbers of milk progesterone analyses to be performed (Hruška et al. 1983). In general, therefore, it can be expected that the number of steroid assays will increase in the coming years.

Traditionally, with the exclusion of estriol analyses in the monitoring of fetal well-being, steroid assay results have not been required rapidly. There are, however, certain situations such as induction of ovulation with or without in vitro fertilization, in which rapid processing of estradiol analyses is imperative, putting demands on the accessibility and speed of the instrumentation used in steroid analyses. Put together, these various trends in the clinical requirements of steroid assays demand the development of more efficient immunological techniques. An essential part has been the development of non-extraction methods for the analyses of, for example, estriol, estradiol, progesterone, cortisol and dehydroepiandrosterone sulfate. This trend has often been associated with the use of coated-tube technology to simplify the separation step in radioimmunoassay by avoiding the use of centrifugation.

The following description of the semiautomated approach used in radioimmunoassays in this laboratory reflects the various needs of being able to accommodate large numbers of different assays, and speed of operation, into a single concept. A large number of other types of approaches for automation or semiautomation of radioimmunoassays has also been described (e.g. Barnard and Collins 1983, Ingrand 1978, Parsons et al. 1983 and references in these).

There is a trend to replace the use of radioactive labels in immunological assays, the reason being to avoid the problems in handling the radioactive waste and to obtain increased shelf-life of these reagents. In assays of the steroids present in low concentrations in the organism, there are problems in obtaining sufficient sensitivity in the assays.