Assessment of Some Hormonal Levels in Egyptian Subjects

Thesis

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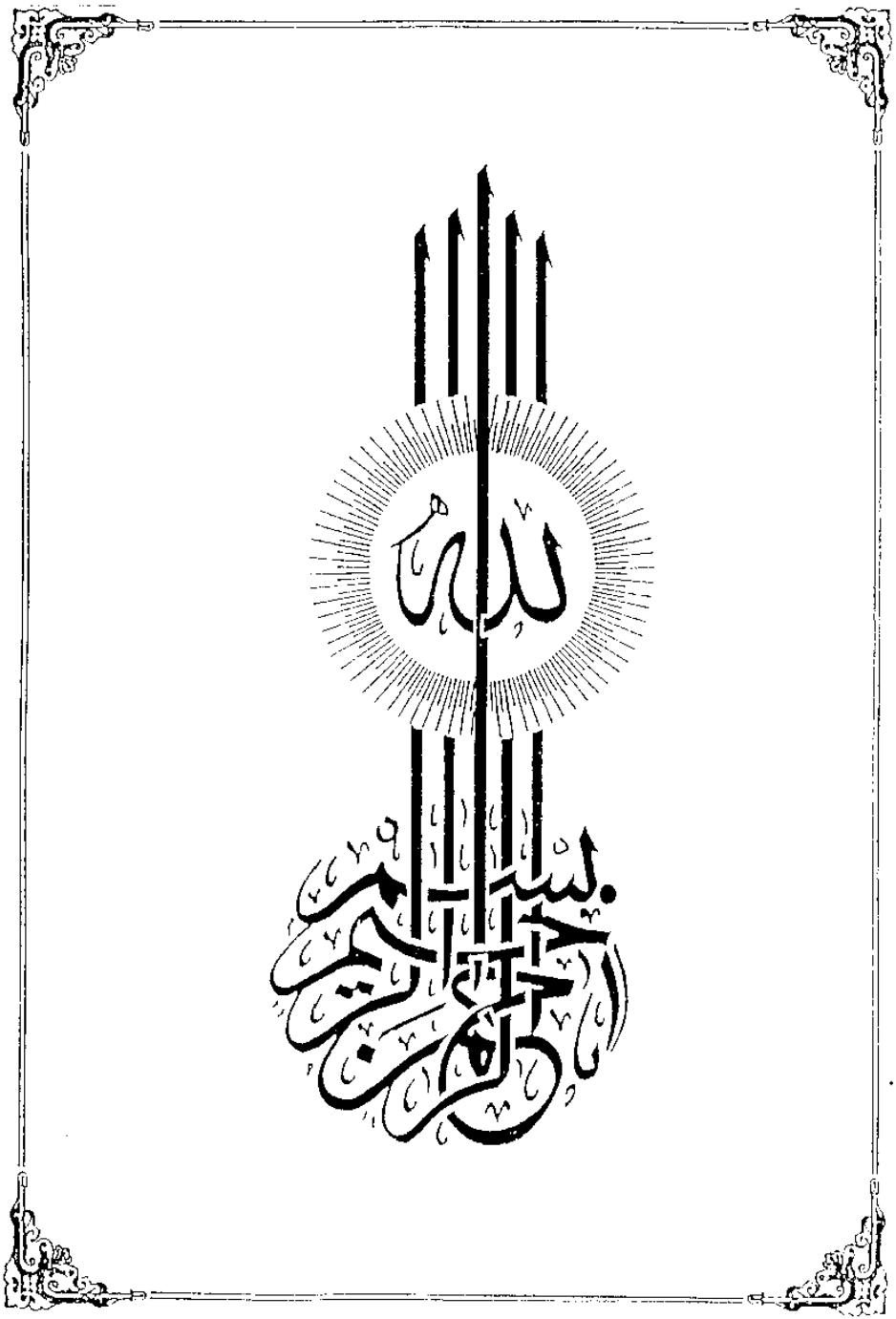

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1.1. INTRODUCTION

In the past three decades, the radioimmunoassay procedure has been applied to a large number of compounds using a variety of classes of specific binding reagents. Good progress of this procedure was achieved after its application to growth hormone by **Greenwood, *et al.* (1963).**

Radioimmunoassay (RIA) is one of displacement analysis **(Robbins and Rall, 1967)** or saturation analysis, **(Ekins 1960 and Grodsky & Forshman, 1960)** which specifies that a limited quantity of antibody is added to an excess of labeled and unlabeled antigen. It is furthermore assumed that the two forms of the antigen compete for binding sites on the antibody, according to the law of mass action **(Day, 1972).**

In another modification of the radioimmunoassay, termed immuno-radiometric assay (IRMA), the antibody is radiolabeled instead of the antigen. This method has several advantages, one being the low assay "blank" values. Furthermore, the antibodies have a uniform general structure, and a high molecular weight. The high stability and relative ease of their being tagged, overcomes some of the difficulties inherent in satisfactorily radiolabeling low molecular weight antigens **(Bristow *et al.*, 1989).**

The RIA technique may be defined as the procedure which measures, virtually, the concentration of any substance. It is now the most widely applied *in vitro* assay procedure for the estimation of compounds in biological fluids. The

thyroid and pancreatic hormones are some of many hormones which could be measured *in vitro* by using the RIA technique.

The determination of thyroid and pancreatic hormonal levels play a crucial role in the diagnosis of various diseases, and different parameters such as age, sex, pregnancy and smoking habits may be involved in the regulation of these hormonal levels.

In the course of the present work, the variations in the levels of thyroid and pancreatic hormones in serum of normal volunteers, due to some physiological status (such as pregnancy, sex and age) and a non physiological factor (smoking) have been studied.

The effect of aging on the deterioration of glucose tolerance was first noted over 70 years ago and there has been an uninterrupted stream of publications since then **(Andres, 1971)**. In spite of the extensive literature available which documents the above observation **(reviewed by Davidson, 1979)**, yet the underlying mechanism(s) has/have not been agreed on, and several questions have been raised in an effort to understand it (them). These questions include the following: Is the age - related decrease in glucose metabolism due to 1) a defect in insulin secretion? 2) decreased peripheral tissue responsiveness to insulin due to a receptor or a post receptor defect? 3) impaired hepatic glucose uptake and/or augmented production? or 4) impaired hepatic suppression of glucose production?.

Many reports have shown that an impaired insulin action is the primary factor responsible for the diminished glucose metabolism in elderly subjects **(De Fronzo, 1979; Fink *et al.*, 1982 and Rowe *et al.*, 1983)**. Since it is well known that, with advancing age, there is a significant reduction in lean body

mass (being approximately 40% between 20-29 yr and 70-79 yr of age) (**Rowe** *et al.*, 1982) and an increase in percent adiposity (**Bjorntorp** *et al.*, 1971), it was suggested that insulin resistance in elder subjects could be explained by this age-related increase in adipose tissue mass (**DeFronzo, 1979**).

Although pancreatic glucagon was suggested to play a critical role in the pathophysiology of diabetes mellitus (**Unger, 1971**), yet little has been reported on age and glucagon physiology. **Berger** *et al.* (1978) reported a rise in fasting plasma glucagon in the third and fourth decades which failed to rise further thereafter. Whereas glucagon concentrations were shown to be significantly lower in the third than in the fourth to seventh decades (**Kalkhoff and Kim, 1979**).

Thus, the present study was carried out to measure fasting insulin and glucagon concentrations in different ages ranging from 3 to 66 years old. Furthermore, fasting and non fasting concentrations of the two hormones were compared in normal males to detect any possible changes in the response of pancreatic B- and A-cells to glycemia with progressive aging. Similarly, the above parameters were studied in normal females to learn whether we could detect any differences in hormonal concentrations between sexes.

Serum levels of thyroid hormones, as a function of age, has been investigated by several authors (**Gaffney** *et al.*, 1962; **Oddie** *et al.*, 1966 and **Ingbar, 1978**). Serum levels of triiodothyronine (T3) in normal individuals have been found to decrease with age (**Rubenstein** *et al.*, 1973) whereas thyroxine (T4) has been found to be unaltered (**Olsen** *et al.*, 1978). Controversial to the above observation, has been the report showing a gradual decrease from childhood to adulthood in T4 level (**Braverman** *et al.*, 1966 and **Ryness, 1972**).

Also, the effect of sex and age on thyroid stimulating hormone (TSH), T3 and T4 has been reported by **Fisher** *et al.* (1977).

Thus, the present study was also carried out to establish such hormonal levels in both sexes, in different age groups in a representative Egyptian population, randomly chosen from rural areas and town dwellers.

Despite a voluminous amount of research directed at elucidating the mechanisms of human carbohydrate metabolism, many unexplored areas remain, especially in the pregnant state. Many studies have made reference to the altered carbohydrate metabolism in pregnancy based upon changes in the oral glucose tolerance test (**Spellacy & Goetz, 1963 and Trayner** *et al.*, 1967). On the other hand, studies using intravenous glucose tolerance tests have shown normal curves during pregnancy and in fact suggest that more insulin may be released by the pancreas during the pregnant state (**Silverstone** *et al.*, 1961). This is in harmony with the early view of **Rosenloecher (1932)**, who reported that there was an increase in the size and number of the islands of Langerhans in the pancreatic tissue of 15 pregnant women.

Many reports indicate that serum thyroxine (T4) levels were elevated without significant variation within the three trimesters (**Schatz** *et al.*, 1968; **Fisher** *et al.*, 1970) while others showed that they were elevated less in the first trimester and plateaued thereafter (**Souma** *et al.*, 1973 **and Rastogi** *et al.*, 1974). The level of T3 on the other hand, was reported to increase with advancing pregnancy (**Hotelling and Sherwood, 1971; Rastogi** *et al.*, 1974 **and Osathanondh** *et al.*, 1976). Whereas **Yamamoto** *et al.* (1979) reported no significant changes in thyroid stimulating hormone (TSH) during the three trimesters.

These controversies clearly indicate a need for the direct measurement of plasma insulin, glucagon and thyroid hormones in pregnant women.

Cigarette smoking is clearly an important risk factor for chronic disease and mortality in the general populations, contributing to the risk of cardiovascular and renal diseases. It is reasonable to suspect, therefore, that cigarette smoking might predispose insulin - dependent diabetic individuals to the development of vascular complications, which are the major cause of death among cases of long duration (**Connell & Louden, 1983 and Dorman & LaPorte, 1985**). Hazards analysis revealed that heavy smoking was a significant independent predictor of all - cause mortality among females but not males (**Moy** *et al.*, **1990**). Further research is thus needed to determine whether a sex interaction is present in other larger population samples.

Benfari *et al.* **(1977) and Saloojee** *et al.* (1982) reported that, potentially important changes in thyroid may occur after cigarette smoking, since several constituents of tobacco smoke possess antithyroid activity.

Moreover, other studies have shown a possible relationship between cigarette smoking and endocrine functions (**Sepkovic** *et al.*, **1984**). Since changes in thyroid hormone concentrations have a profound effect on overall metabolism as well as influencing both androgenic and estrogenic steroid activity (**Virion** *et al.*, **1980**), thus, the relationship between cigarette smoking and thyroid function has been investigated in the present study.

1.2. AIM OF THE WORK

The determination of hormonal levels play a crucial role in the diagnosis of various diseases and the anticipation of future complications as well as the following up of different therapies and the detection of possible abnormalities in some vital processes of the body.

Many factors are involved in the regulation of the pancreatic and thyroid circulating hormone levels, namely insulin, glucagon, triiodothyronine (T3), thyroxine (T4) as well as thyroid stimulating hormone (TSH).

This work is an attempt to study some of these factors and to evaluate the normal levels of the previously mentioned hormones, under different physiological (age, sex and pregnancy) and non physiological factors (smoking).

1.3. LITERATURE REVIEW

1.3.1. Hormonal Assays

Prior to 1960, it was exceedingly difficult to measure substances that were present in small amounts in blood and other fluids. Until then, chemical determination and bioassay of these substances - usually hormones - were somewhat crude, leading to considerable inaccuracy in measurement of such small amounts.

A further drawback was the difficulty to reproduce the obtained results in other laboratories. In addition, rather large samples were usually needed for these determinations.

Immunoassay is the quantitative measurement of a substance of interest (analyte) using antibodies which bind specifically to that analyte. These two components of the assay will be referred to as "ligand" and "antibody" respectively. In order to perform such an assay, it is necessary to measure the binding of the ligand to the antibody at very low concentrations and this has most commonly been achieved with the use of radioisotopes. The first practical application of the principle was the technique of radioimmunoassay, invented by **Yalow and Berson (1960),** for the quantitative measurement of insulin based on a new technique of competitive protein binding assay (CPBA).

At approximately the same time, **Ekins (1960),** reported a similar method for the determination of plasma thyroxine concentrations, which was also based on a competitive binding principle.

This technique, and subsequently developed related techniques such as the immunoradiometric assays (IRMA) and enzyme-linked immunosorbent assays (ELISA), have revolutionized the biological sciences. Using these methods, the minute quantities found in biological materials of a whole range of analytes such as protein hormones, steroids, viral antigens, cytokines, and clotting factors could be accurately measured without relying on difficult and sometimes imprecise measurements based on their biological activities. Indeed, it is hard to overestimate the impact that the development of immunoassays has had on the life sciences.

Although the basic principles of the radioimmunoassay (RIA) and of the immunoradiometric assay (IRMA) have remained the same, both methods have been the subject of countless refinements and improvements, some of which have included the use of non-isotope labels (enzymes, luminescent labels), the development of novel method of separating free from bound antigen such as magnetic particles, or quantifying that ratio without separation (e.g. fluorescence depolarization) and the use of specific combinations of monoclonal antibodies

The theoretical basis of immunoassays employing isotopes have been reviewed by Ekins *et al.* (1976). Two basic types of immunoassays can be recognized: limited reagent assays and reagent excess assays, the two basic methods being typified by RIA and IRNA respectively

Limited reagent assays have been described by a number of terms such as "saturation assay" and "displacement assay". Limited reagent assays employing isotopes and antibodies are almost invariably referred to as "radioimmunoassay" a somewhat unfortunate term since it does not, in any way, describe the theoretical basis of the assay method.

The underlying principle in all "limited reagent" assays is that by limiting the concentration of one of the reagents, the system is saturable. In the technique of RIA, a radiolabeled ligand reacts with antibodies which bind specifically to that ligand. The amount of antibody available to react with the ligand is limited such that it is saturable, only a fraction of the total labeled ligand is bound to the antibody. The reaction may be written as:

$$Ab + {}^*L \longrightarrow Ab{}^*L$$

where Ab = antibody, and *L = radiolabeled ligand.

If the reaction is allowed to reach equilibrium in the presence of a ligand that is not radiolabeled, two simultaneous reactions will take place;

$$Ab + {}^*L \longrightarrow Ab{}^*L$$

$$Ab + L \longrightarrow AbL$$

Where L = unlabeled ligand.

Under limited antibody conditions therefore, L will compete with *L for available antibody, and the fraction of radiolabeled ligand that is antibody-bound will fall as the amount of unlabeled ligand present increases. In practice, the fraction of antibody-bound label is measured using a system that separates bound and free ligand. Increasing concentrations of unlabeled ligand generate a displacement curve of the type shown in Figure (1-1).

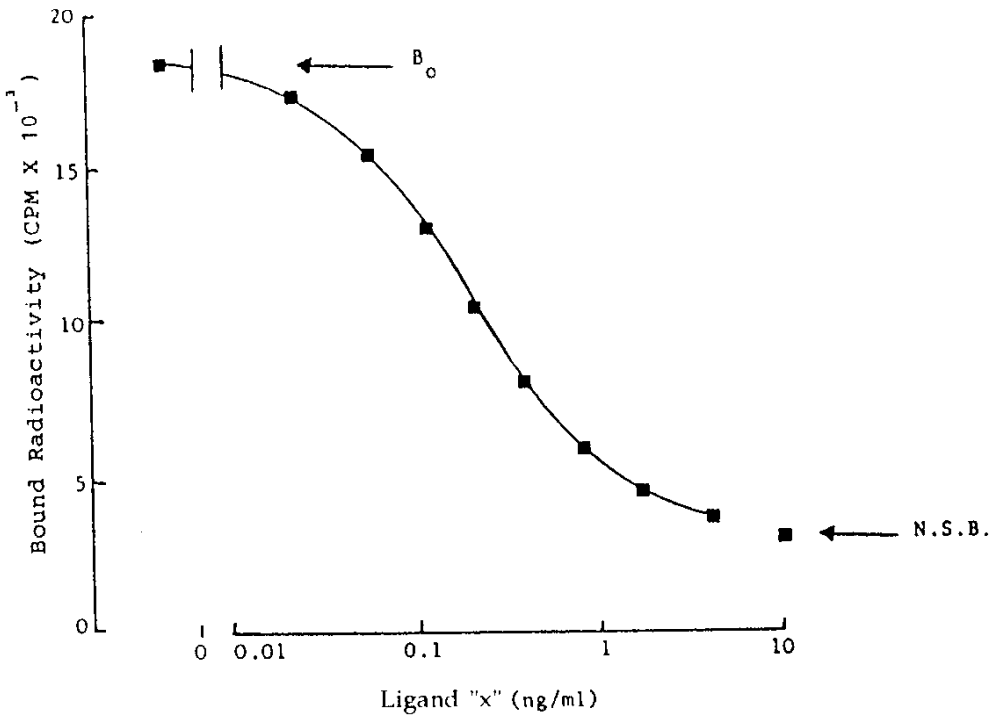

Fig. (1-1): Radioimmunoassay of ligand "x". B_o = per cent binding at zero ligand concentration. NSB (non-specifici binding) = per cent binding at zero antibody concentration.

In reagent excess immunoassays, the antibody is present in excess, such that at equilibrium all available ligand is antibody-bound, and the majority of antibody remains unused. In practice, the amount of antibody that has ligand bound to it is determined. Measurement of antibody-ligand complex in this type of assay is most conveniently achieved by radiolabeling the antibody, the ligand-bound radiolabeled antibody is then separated from the free radiolabeled antibody by some convenient procedure. The most widely used immunoassay based on the excess reagent principle is the immunoradiometric assay (IRMA). In the first IRMA procedures to be described, the free labeled antibody was