OPTIMIZATION OF INSULIN RADIORECEPTOR ASSAY IN HUMAN ERYTHROCYTES IN NORMAL AND SOME DISEASE STATUS

THESIS
Submitted By

ABDEL - FATTAH MAHMOUD AHMED

(B. Sc. 1981 and M. Sc. 1989)
in Biochemistry

FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY OF SCIENCE
IN
BIOCHEMISTRY

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DEPARTMENT OF BIOCHEMISTRY
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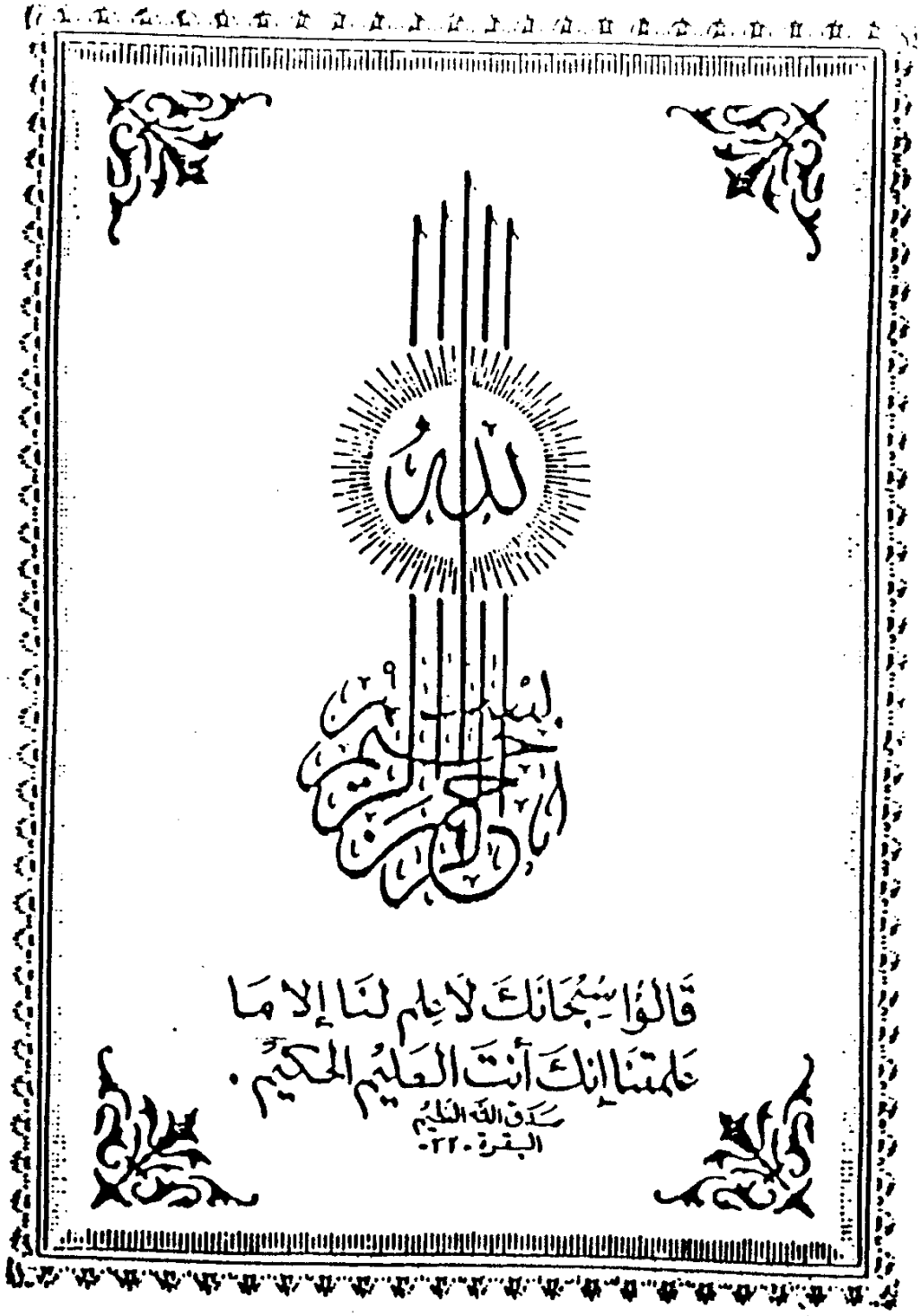

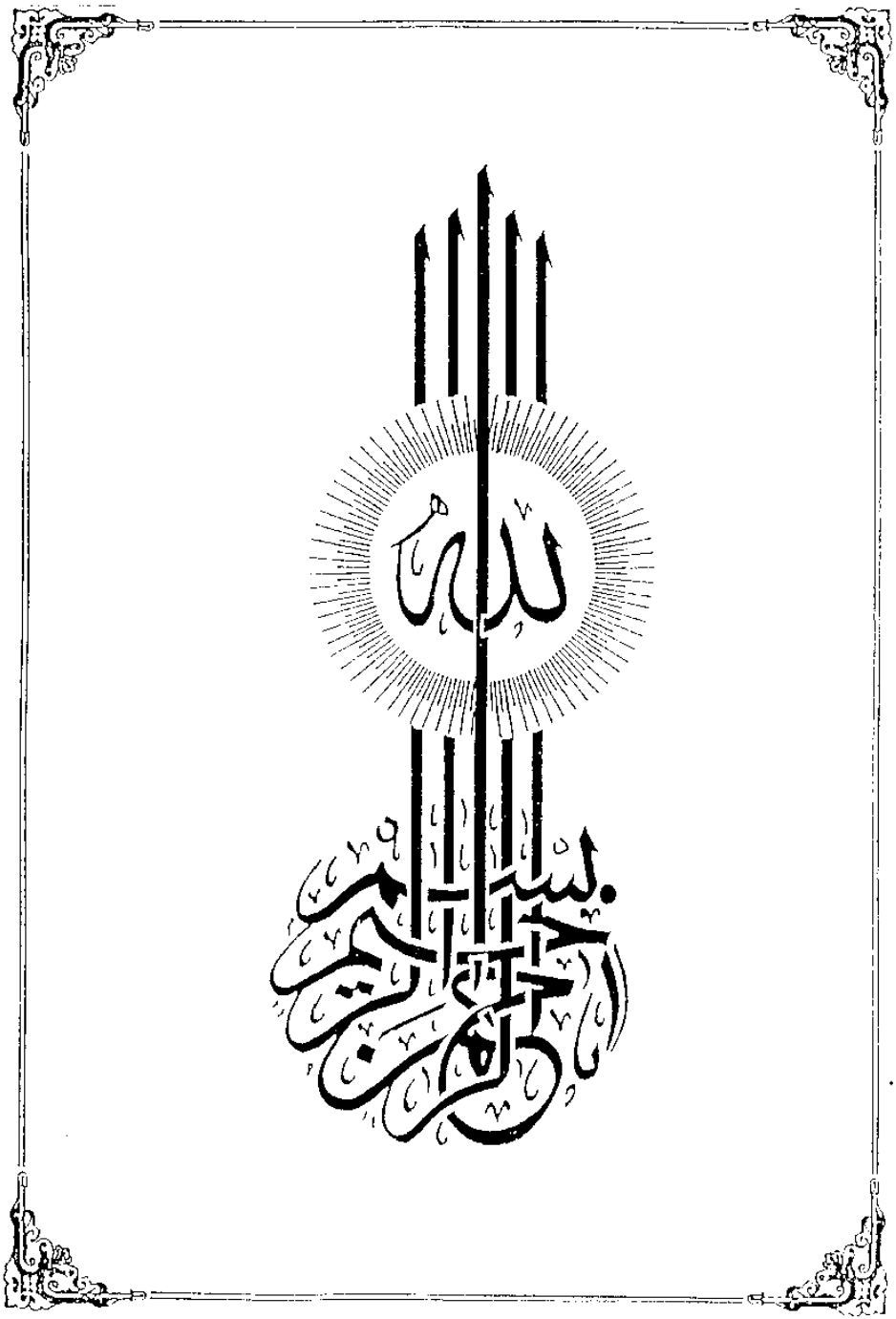

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TO MY FAMILY

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A B S T R A C T

This study is concerned with the evaluation of a new optimized technique for the principle of chloramine-T method used for insulin iodination by ^{125}I-radioisotope with some modifications. The modified procedure can be carried out under normal condition of room temperature , employed longer reaction times and omitted the addition of inorganic reducing salts, maintaining efficient iodination and avoiding denaturations to obtain labels of exccedingly high specific activity on a small quantities of insulin for in vitro usage in the investigation of human erythrocytes ^{125}I-insulin binding capacity in normal and some disease status.

C O N T E N T S

INTRODUCTION

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

Yalow and Berson (1960), reported a method for the quantitative measurement of insulin based on a new technique of competitive protein binding assay. 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.

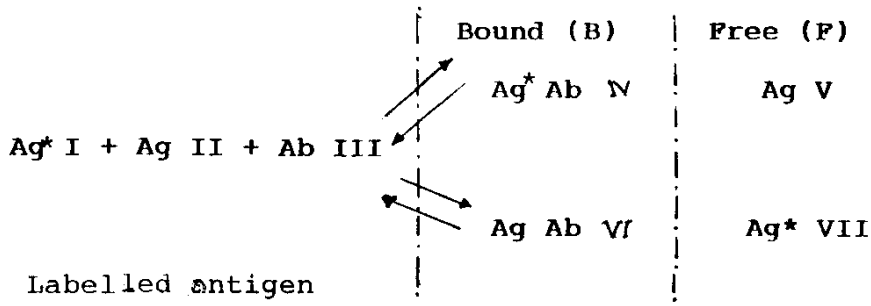
Scince 1960, new methods have been widely applied as the radioimmunoassay (RIA) technique for the quantitative estimation of many substances, particularly hormones.

Radioimmunoassay is one of displacement analysis (Robbins and Rall, 1967). or saturation analysis (Grodsky and Forshman, 1960 and Ekins et al., 1970). which specifies that a limited quantity of antibody is added to an

excess of labelled antigen, and unlabelled 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 et al., 1972).

The labelled antigen and unlabelled one may also be referred to as the first molecule and the antibody as the second molecule, or specific reactor (Korenman, 1968). Unknown concentrations of antigen may be determined by taking advantage of the observation that the radiolabelled molecules, or tracer compete phyisicochemically with the nonlabelled antigen molecules, either standards or unknowns for binding sites on the antibodies (Yalow and Berson, 1959).

Radioimmunoassay has several advantages as it is cheaper and quicker, thus it remains less costly in performing hundreds or thousands of assays. It is more sensitive, owing to its higher affinity constants, as well as, the nature of the "specific reactor", (the antibody) in radioimmunoassay offers greater specificity. The antigen-antibody interaction is generally accepted that the reaction reaches an equilibrium during the incubation procedure, according to the following formula, which discusses the basic kinetics of radioimmunoassays (Potts et al., 1967).



I Ag* : Labelled antigen

II Ag : Unlabelled antigen

III Ab : Antibody to Ag* and Ag

IV Ag*Ab : Labelled antigen bound to antibody (B)

V Ag : Free unlabelled antigen

VI AgAb : Unlabelled antigen bound to antibody

VII Ag* : Free labelled antigen (F)

$$Ag + Ab \underset{K_1}{\overset{K}{\rightleftharpoons}} Ag\,Ab \quad \ldots\ldots\ldots \quad (1)$$

where K is the rate constant for association and K_1 is the rate constant for dissociation.

At equilibrium :

$$K\,(Ag)\,(Ab) = (AgAb) \quad \ldots\ldots\ldots \quad (2)$$

Also, the ratio of the bound antigen (B) to free antigen (F) is :

$$B/F = (AgAb)/(Ag).$$

It is assumed that, the equilibrium constants for the unknowns, are equal. Varying conditions (e.g. during the incubation) may affect each equilibrium constant, differe-ntly, and the antibodies may discriminate between labelled and unlabelled antigens.

The presentation of statistical controls for radio-immunoassay has been evaluated (Midgley et al., 1969 and Vivian and La Bella, 1971). and a number of calculator , and computer programs have been written for the analysis, description and simulation of equilibrium behavior of the complex immunoreactive systems involving antigens and anti-bodies (Midgley et al., 1971).

Radioimmunoassay techniques may be defined as the procedure which measure virtually the concentration of any substance. It is now the most widely applied in vitro assay procedure for the estimation of compounds in biolo-gical fluids of interest.

Insulin is one of many hormones which can be measured in vitro using radioimmunoassay techniques.

The discovery of insulin by Banting and Best represents one of the major huminitarian and scientific milestones of