RADIOIMMUNOASSAY OF SERUM INSULIN
BY PHADEBAS INSULIN TEST

Vachira Mokamol, M.D. *

Muni Keoplung, M.D. *

Jit Jiraratsatit, M.D. *

INTRODUCTION :

Various methods have been used to determine serum insulin level. However, most of these methods are perplexing and not suitable for clinical use. Until the introduction of radioimmunoassay techniques which offer high sensitivity, specificity and simplicity more than other biological methods, the clinical application, then, is widespread. In common, radioimmunoassay methods depend on ability of an antibody to bind its antigen which is labelled with radioisotope and its competitive inhibition of this reaction by the unlabelled antigen. The antibody-bound labelled and unlabelled antigen is separated from the unbound and the radioactivity in one or both of these two fractions is measured. Therefore, the reproductivity and accuracy depend on the purity of any specific antibody and completeness of its reaction with the antigen.

The purpose of this study is to assess the simplicity and accuracy of the " Phadebas " insulin test before it is accepted as a part of our clinical laboratory study and to determine the serum insulin levels in non-diabetic subjects.

MATERIAL AND METHODS :

Nine of the patients who were admitted to the medical and surgical wards of non-diabetic and non-pancreatic disease, were selected for study. The ages ranged from 14 to 61 years; all were male. They are Thais of same socio-economic level. Before and during the study period, they were allowed to eat three ordinary meals

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* Department of Medicine, Faculty of Medicine, Chiang Mai University.

a day which were rather large and high in carbohydrate. The blood samples were drawn at 6:45, 9:00, 11:45 a.m., 2:00, 16:45 and 7:00 p.m. that was about 15 minutes before and 2 hours after each meal. The serum was separated and, as soon as possible, a part of serum was sent for glucose analysis, the other was kept frozen until the determination of insulin was done. Technicon-Autoanalyzer was used for the determination of serum glucose and "Phadebas" radioimmunoassay insulin kit was employed for the determination of insulin. The detailed principles and technical procedures are described in the manufacture's instruction manual. (1,2)

RESULT:

The table showed age; serum insulin and sugar values at various periods. The control fasting levels of insulin ranged from 8-112 Uu/ml (mean = 36,7). The maximum mean insulin output was at 9.00 a.m. that was 2 hours after breakfast. The control fasting serum sugar ranged from 70-95 mg% with the mean of 85.6. It reached highest peak (mean = 112.50) at 7:00 p.m.

The relationship between the serum insulin and serum sugar during the 12 - hour study periods was shown in the figure. The upper graph represented the serum glucose and the lower graph represented serum insulin. Obviously, the slopes and regression lines were almost entirely reconcilable except that at 7:00 p.m. where the serum sugar made an increase whilst the serum insulin made a fall.

DISCUSSION:

The radioimmunoassay methods enable one to determine very low concentrations of insulin in rather small samples of serum. As claimed by the manufacterer, the "Phadebas" insulin test offers a simple and accurate method for insulin determinations. It is practical and good results can be obtained for up to 4 months of proper storage at 2-8°C. After reconstitution, the I ^{125} insulin and insulin standard shelf life and storage is about one week at 2-8°c.

Its principle is described as followed: "An aliquot of the sample to be determined is mixed with a given amount of iodinated insulin (insulin I ^{125}) and a certain amount of an immnosorbent consisting of Sephadex particles to which insulin antibodies have been coupled (Sephadex-Anti-insulin complex). After incubation, its particles are sedimented by means of centrifugation and washed, the radio activity bound to the Sephadex particles is measured. The radioactive uptake on the immunosorbent varies inversely with the quantity of unlabelled insulin present in the original mixture".
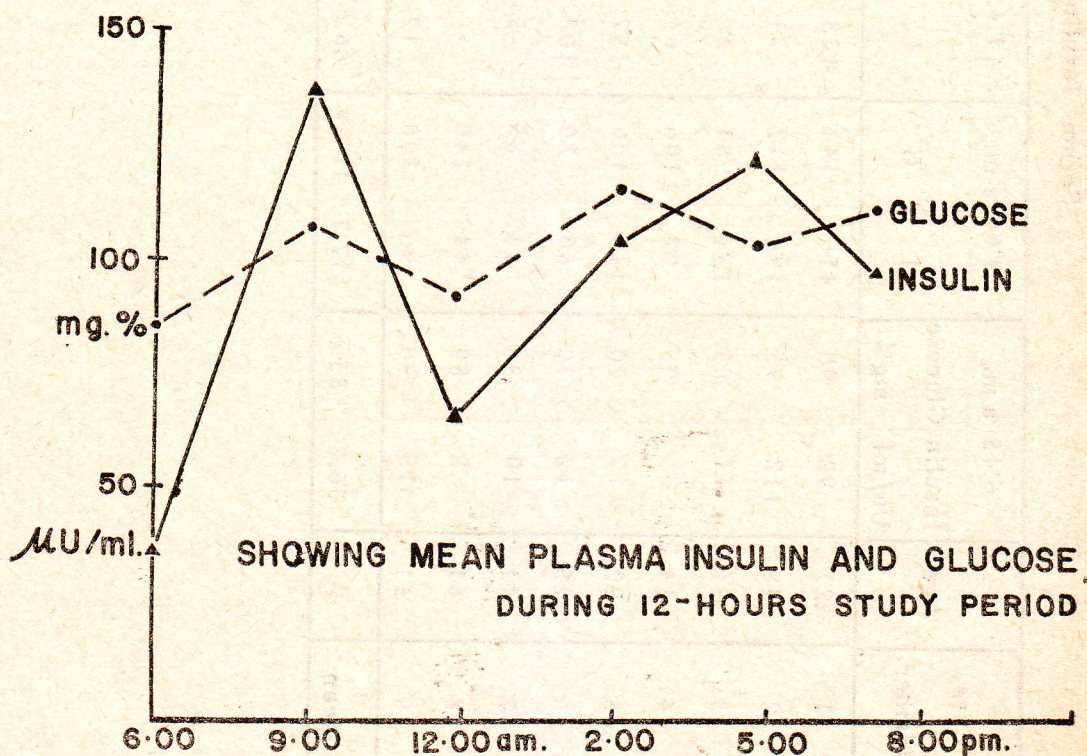
This is not a time consuming but a rather simple method which requires less special instruments. However, our rrsults are not as what we expected. More study should be done before it is definitely claimed. The volume of I^{125} insulin offered, seems to be unproportionally smaller than the rest of reagents. The price per test is economical but to use the whole lot of reagents in one gronp of study may lower to cost and avoid reconstitution of reagents, repeat freezing and thawing of insulin standard and insulin I^{125}.

The result of insulin secretory pattern seems to relate with meal and serum glucose except pre and post last meal period. It is not comparable with the pattern found in European people. Dietary pattern of having large three meals a day and some technical error should be considered.

SHOWING MEAN PLASMA INSULIN AND GLUCOSE DURING 12-HOURS STUDY PERIOD

Serum Insulin and Glucose

Case Number	Age	6:45 a.m. Insulin Uu/ml	6:45 a.m. Glucose mg%	9:00 a.m. I	9:00 a.m. G	11:45 a.m. I	11:45 a.m. G	2:00 p.m. I	2:00 p.m. G	4:45 p.m. I	4:45 p.m. G	7:00 p.m. I	7:00 p.m. G
1	21	92	90	416	141	128	94	108	130	120	96	184	117
2	48	112	95	194	112	24	87	110	94	154	104	76	134
3	31	—	85	154	81	48	100	96	110	108	100	296	93
4	14	—	95	92	106	64	117	184	127	—	97	196	112
5	22	17	70	140	106	152	114	87	104	124	112	33	106
6	25	12	70	60	80	100	88	72	112	25	100	31	118
7	41	10	86	43	85	5	80	102	112	17	114	13	106
8	61	8	89	44	140	—	76	—	132	352	102	54	146
9	41	15	90	96	100	12	88	76	126	80	112	9	90
Mean		36.7	85.6	137.7	107.9	66.6	93.8	104.4	116.3	122.5	104.0	99.0	112.5

REFERENCES

1. Phadebas insulin test instruction manual Pharmacia AB, uppsala, Sweden. 1972 : Phadebas Insulin Test Instruction manual.

2. Simultaneous Glucose/BUN Method, Technicon Autoanalyzer, 1967.

ย่อเรื่อง

ได้ทดลองตรวจหาระดับของ serum insulin ในคนที่ไม่มีโรคเบาหวาน หรือโรคของตับอ่อน 9 คน เป็นชายทั้งหมด มีอายุตั้งแต่ 14–61 ปี โดยใช้ "Phadebas" radio-immunoassay insulin kit พบว่าจำนวนของการหลั่งของ insulin เปลี่ยนแปลงไปตามอาหารแต่ละมื้อ แต่รายละเอียดอื่น ๆ ไม่อาจใช้เปรียบเทียบ กับ ผล ของ การ ทดลองใน ต่างประเทศ ทั้งนี้เพราะจำนวนยังน้อย และลักษณะอาหารที่ใช้บริโภคก็แตกต่างกันมาก

น้ำยา kit แบบนี้ใช้ง่ายสะดวก และถ้าจัดตรวจพร้อม ๆ กันหลาย ๆ คนเพื่อจะได้ใช้น้ำยาทั้งชุดเลย ก็จะเป็นการทุ่นค่าใช้จ่าย และป้องกันการเสื่อมค่าของ insulin ด้วย รายละเอียดอื่น ๆ ต้องรอผลของการ ศึกษาต่อไป.