

# PLASMA INSULIN ESTIMATION AND ITS SIGNIFICANCE IN DIABETES MELLITUS

By

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The concept of insulin deficiency as the only cause of diabetes mellitus was questioned when certain diabetics showed resistance to insulin. To prove or to disprove this hypothesis it was necessary to estimate the plasma insulin level in normal and diabetic persons. Since 1938 various workers have tried to achieve this goal. The earlier 'intact animal methods' have now given place to a more sensitive 'rat diaphragm method'.

The rat diaphragm method measures the effective insulin level of the plasma (Vallance-Owen and Hurlock, 1954) which is the sum total of the activity of insulin and its antagonists. Recently Baird and Bornstein (1957) employed an elaborate method to extract insulin free from the anti-insulin factors. Takeuchi *et al* (1957) and Wright (1957) reported that dilution of plasma gave higher insulin values. We used diluted plasma to test the insulin effect with a view to obtain the absolute insulin concentration of the plasma.

## MATERIAL AND METHODS

The work was carried out on diabetic patients admitted in the Sassoon Hospitals Poona under our care. Insulin was discontinued for 48 hours and Carbutamide for 15 days before the assay of plasma insulin. Each patient was starved overnight and 10 ml. of venous blood was collected in a heparinised centrifuge tube in the morning while the patient was still in bed. 1 ml. of this blood was used for glucose estimation by Folin's method and the rest was centrifuged to separate the plasma.

Normal subjects were healthy patients admitted into the hospital for operations such as hernia. Post-prandial blood glucose was estimated in each case to exclude any latent derangement in the carbohydrate metabolism. The same procedure of collection and utilisation of blood was followed as in the diabetics.

Rats weighing between 100 and 150 Gms were starved for 24 hours, killed by stunning, hemidiaphragms removed quickly, their serrated edges

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trimmed, and placed in the appropriate beaker previously immersed in ice. Each beaker contained two hemidiaphragms from two different rats, and in addition either medium alone, medium containing insulin, or plasma medium mixture as detailed below.

The incubation was carried out in the Dubnoff Metabolic Shaking Incubator at 38°C with 100 per cent oxygen as gas phase and shaking rate of 60 oscillations per minute for 2 hours. At the end of the incubation period the diaphragms were blotted dry between filter papers and weighed. Glucose in the incubated and respective unincubated control samples was estimated. The difference between the two was taken as the uptake of the rat diaphragm which was calculated as mg. per Gm. wet weight of the diaphragm per hour. The increase in the glucose uptake produced by insulin was plotted on the graph against the Log. of respective insulin concentration in the medium. The increase produced by the plasma was marked on this graph and insulin concentration read therefrom. Since plasma was diluted ten times this value was multiplied by ten.

Medium used was Stadie and Zapp (1947) phosphate buffer containing 0.2 per cent glucose at pH 7.4. The plasma-medium mixtures were prepared by mixing 1 ml. of plasma with 9 ml. of medium. The insulin medium mixtures were prepared with crystalline plain insulin (Boot's). Usually three insulin concentrations between  $10^{-2}$  and  $10^{-5}$  unit per ml. were used in one experiment to plot the graph on which the value of the plasma was fixed. Usually one and occasionally two plasma insulin estimations were done at a time. Quantity of incubation fluid was 2 ml. in each case.

#### RESULTS

Table 1 gives the details of each patient. Plasma insulin was estimated in five nondiabetic adults and ten diabetic patients. The plasma insulin levels in five nondiabetics ranged from 0.7 to 6.3 milliunit per ml. In two middle aged diabetics whose disease was discovered less than six months previously and who did not require insulin, the plasma levels were 10 m unit per ml. and 3.1 m unit per ml. which is in our normal range. Four middle aged diabetics who required insulin and who responded favourably to BZ-55 or D-860 had a concentration between 0.1 m unit per ml. to 0.31 m unit per ml. which is less than normal but more than that of two other diabetics in the same age group whose values were 0.015 and 0.039 m unit per ml. The former of these latter two failed to respond to BZ-55. Plasma insulin concentration of the two diabetics in the younger age group were 0.25 and 0.039 m unit per ml. respectively. A 1 in 2 dilution of their plasma actively depressed the glucose uptake of the rat diaphragm probably due to the excess of anti-insulin factors in their plasma.

TABLE I

## PLASMA INSULIN OF DIABETICS AND NORMAL PERSONS

NO	AGE YRS	SEX	DURATION OF DISEASE	INSULIN REQUIREMENT	PLASMA INSULIN M UNITS/ML	F. B. S.		GLUCOSE UPTAKE	
								WITHOUT PLASMA	PLASMA 1:2 DILUTION
1	27	M	OVER 3 YRS	30 U B.D.	0.25	296	ANTI INSULIN	3.8	2.0
2	20	F	?	40 U B.D.	0.039	360	FACTORS ++	3.3	2.2
3	60	M	OVER 2 YRS	50 U B.D.	0.015	316	DID NOT RESPOND TO BZ 55		
4	40	M	2 YRS	30 U B.D.	0.039	204			
5	50	F	1 YR	+	0.31	220			
6	50	F	10 YRS	30 U B.D.	0.10	266	GOOD RESPONSE		
7	40	M	2 YRS	+	0.12	280	TO BZ 55 OR DB60		
8	45	F	?	+	0.25	190			
9	48	M	6 MONTHS	NIL	10.00	144	NO INSULIN OR		
10	54	M	6 MONTHS	NIL	3.10	100	BZ 55 NEEDED		
11	50	M	-	-	6.30	91			
12	52	M	-	-	0.70	106	NORMAL ADULTS		
13	47	M	-	-	6.30	101			
14	65	M	-	-	3.10	98			
15	48	M	-	-	0.79	88			

FIG. I

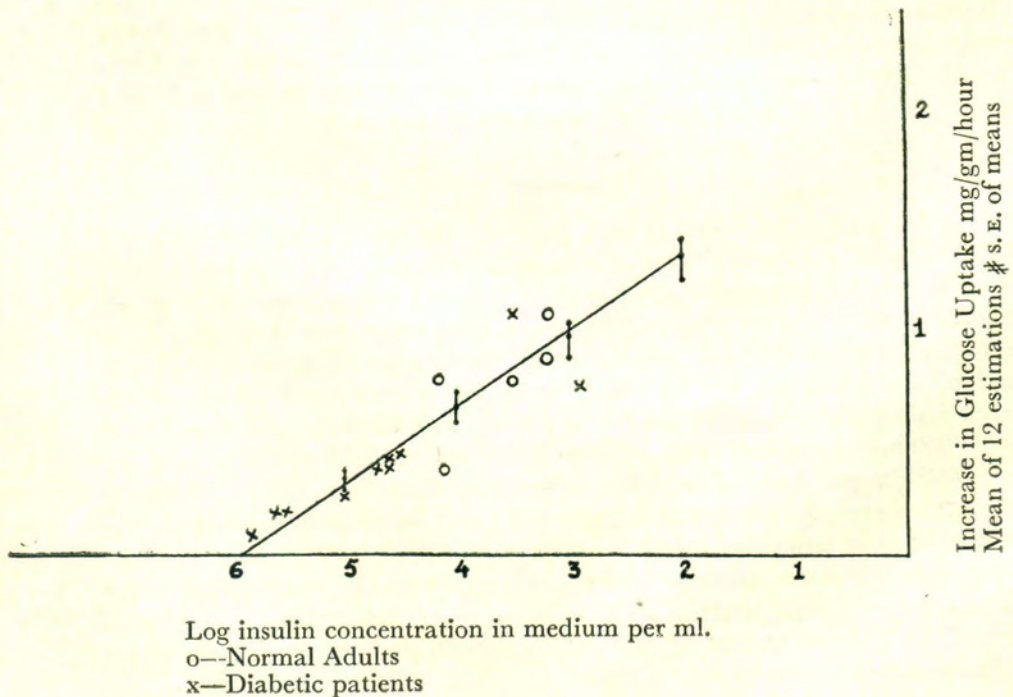


TABLE II  
 NORMAL PLASMA INSULIN CONCENTRATION AS REPORTED BY  
 VARIOUS WORKERS.

Worker	Medium & Plasma Dilution	Normal Insulin Concentration Micro Unit/ML.	Reference
Groen et al	Gey & Geys. 1 : 6	62.5 to 625	4
Randle P. J.	Gey & Gey's Medium 1 : 4	9000 to 22000	7
Vallance-Owen & Hurlock	Gey & Gey's Medium. Undiluted Plasma	40 to 80	10
Baird & Barnstein	Extract of Plasma	1000 to 2000	2
Peter Wright	Gey & Geys Medium. Undiluted Plasma	40 to 80	12
Aiman & Kulkarni	Stadie's Medium. Dilution 1 : 10	700 to 6300	This Report

Fig. 1 is the graph of the increase in the glucose uptake against the Log. of the respective insulin concentrations (the mean of 12 estimations with S. E. of means) and showing the plasma insulin concentrations of the normal and the diabetic persons.

Table II gives the normal plasma insulin values as reported by various workers.

#### DISCUSSION

The pathogenesis of diabetes mellitus which is still obscure has necessitated the estimation of plasma insulin. Bornstein and Lawrence (1951), using A.D.H.A. rats came to the conclusion that Juvenile diabetics do not have insulin in their plasma whereas middle aged diabetics have it. Anderson *et al* (1947) demonstrated that plasma of juvenile insulin resistant diabetics contained anti-insulin factor (s). Groen *et al* (1952) first used the rat diaphragm method for plasma insulin estimation. This method has been further modified by various workers in order to increase the sensitivity and reliability. Vallance-Owen & Hurlock (1954) thought that by using undiluted plasma they could get higher insulin concentration but in fact got similar value as those of the previous workers. Randle (1954c) using 1 in 4 dilution of the plasma obtained higher values. Takeuchi *et al* (1957) reported that dilution of plasma dilutes insulin antagonising factors to an ineffective level and it could be used to determine the plasma insulin levels of insulin resistant diabetics. Wright (1957) repeated the procedure of Vallance-Owen and confirmed his findings. He also confirmed that dilution of plasma yields higher insulin values.

From work reported so far it is clear that juvenile diabetes is due to the excess of anti-insulin factors circulating in the plasma, whereas diabetes of middle age is more often due to deficiency of insulin in the plasma. During growth large amounts of insulin are released under the influence of Growth Hormone. Hence in juvenile diabetes it is important to know whether large excess of growth hormone circulates in the plasma, leading, in the initial stages to higher than normal plasma insulin concentration (as in acromegaly—Randle 1954a) and proceeding in later stages to exhaustion of pancreas, or whether pituitary function is normal but pancreas for some reason fails to respond by releasing extra insulin needed for nitrogen retention. Single plasma insulin estimations are, therefore, insufficient. Repeated estimations at regular intervals throughout the growing period of many subjects—normal and diabetic—should be done. Both the effective and actual insulin levels either by diluting the plasma or by using some extraction method should be determined. At the same time efforts will have to be made to determine the exact nature of anti-insulin factors.

Similar data in middle aged diabetics may reveal progressive decline in plasma insulin levels. We noted normal plasma insulin in two patients whose

disease was very recently diagnosed and who required no insulin for stabilisation. Wright (1957) has reported similar findings. He also reported normal rise in plasma insulin after ingestion of glucose (fed) in these cases. This is an interesting finding and suggests that insulin release from  $\beta$  cells occurred only at raised threshold of hyperglycemia. This necessitates the study of factors such as blood glucose levels, growth hormone, adrenal cortical hormones, and glucagon which might influence the release of insulin in normal and diabetic persons.

Another possible use of this method is in screening patients for sulphonylurea therapy. In our series, those patients who had moderate concentration of insulin in their plasma responded favourably, whereas one whose plasma contained negligible insulin failed to respond. Our figures are too small and further work is contemplated in our laboratory. Groen *et al* (1952) suggested the use of this method in differentiating functional hypoglycemia from hyperinsulinism.

In conclusion, we believe that the rat diaphragm method should be explored for the better understanding of pathogenesis of diabetes mellitus. As Randle (1954c) has said there is considerable variation in the normal insulin levels as reported by various workers (Table II). We have been trying to study the insulin levels by this method. Our data is small and there is considerable scatter in the normal insulin concentration. We are aware that much more work will have to be done on this subject. We report our work here only to stress the need and the scope of such a study.

#### SUMMARY

Plasma insulin levels have been determined by the rat diaphragm method in five normal and ten diabetic persons. Two diabetics in the older age group who could be stabilised on diet alone had normal fasting plasma insulin values. Two diabetics in the younger age group had an excess of anti-insulin factors in the plasma. The possibility of correlating plasma insulin values with the success or otherwise of the sulphonylurea therapy has been suggested. The significance of plasma insulin estimation in elucidating the pathogenesis of diabetes mellitus has been discussed.

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