CHAPTER - IX

SEASONAL ALTERATIONS IN GLUCOSE TOLERANCE AND INSULIN RESPONSE OF NORMAL, SHAM OPERATED AND PINEALECTOMISED WILD PIGEONS, COLUMBA LIVIA

Pancreas is the principal endocrine gland involved in maintaining carbohydrate homeostasis in vertebrates. Additionally, pituitary and adrenal and even thyroid to some extent are known to exert influence on carbohydrate metabolism. Hormonal principles of these glands together with glucagon of pancreas are essentially involved in elevating the glycemic level. The role of insulin in lowering the glycemic level in opposition to the above factors is a commendable singular action. Amongst the amniotes the relative importance of the two pancreatic hormones, insulin and glucagon vary considerably. Whereas same reptiles show dependance on insulin and others on glucagon, the aves have evolved into total glucagon dependance and the mammals into total insulin dependence (Miller and Lagios, 1970; Hazelwood, 1973). Obviously, the mammals elicit greater sensitivity to insulin while the aves respond better to glucagon. This differential adaptation is manifested in the form of tolerance to higher blood glucose loads in the case of birds.

Of late, the pineal in different vertebrate species has been noted to influence glycemic level as well as the glycogen reserves of the body. Based on the studies on this line some sort of pineal-pancreas axis is considered a likely entity in regulating the carbohydrate metabolism. Interpretations based on the results obtained seem to project a differential relation of this axis. Accordingly, Mihail and Guirgea (1979) have suggested pineal to be capable of compensating for the lack of endocrine pancreas, while Csaba and Barath (1971) had suggested a suppressive influence of pineal on the β cells of pancreas in rats. Based on the previous studies from this laboratory, Patel (1982) had considered pineal in wild pigeons to be anti-insulinic. Moreover, this relationship between pineal and insulin was also shown to be more season specific. Studies from other laboratories also support the contention of season specific influence of pineal on carbohydrate metabolism (Delahunty et al., 1978). It was with a view to gain more confirmatory evidence on the earlier observation of an anti-insulinic role of pineal, that glucose tolerance test (GTT) and insulin response test (IRT) on blood glucose of normal, sham operated and pinealectomised wild pigeons have been undertaken on a seasonal basis vis a vis breeding.

MATERIAL AND METHODS

Adult feral blue rock pigeons of both sex were procured (during both the breeding and non breeding months) from the local dealer and maintained in the laboratory on natural photoperiodic and temperature conditions with grains as food and supply of water ad libitum. After acclimation, the birds were divided into three groups of 30 each i.e. intact controls (C) sham operated (PN) and pinealectomised (PX). Pinealectomy was performed as described previously and 30 days post-surgery PX birds along with the corresponding controls (PN and C) were used for GTT and IRT. The blood glucose was estimated by the micromethod of Folin and Malmros (1929). GTT: Birds of all the three groups were fasted overnight and infused intravenously with glucose, 70 mg/100 gm body weight per bird. Blood samples were drawn at 0, (Prior to glucose loading) and 30, 60, 90 and 120 minutes post-glucose loading for evaluation of blood glucose. IRT : Insulin response was assessed by injecting 1.5 IU insulin/bird (in 1 ml of 0.9 % saline) and the resultant hypoglycemia with recovery to normal glycemic level were measured by drawing blood samples at fixed time intervals of 30, 60, 90, 120, 150, 180, 210, 240, and 270 minutes after insulin injection. The normal glycemic level was measured from blood samples drawn prior to insulin injection. The insulin preparation used for the study was of Boots Company (India) Ltd. containing 40 units/ml.

RESULTS

GTT AND IRT DURING THE BREEDING SEASON

The fasting blood glucose prior to glucose infusion was 216.00 mg/100 ml and 190.00 mg/100 ml in birds of C and PX groups respectively. The birds of PN group had an intermediate level of 199.99 mg/100 ml. After glucose infusion, the glycemic levels rose to their maximum in 30 minutes. The increase was to the extent of 11.49 % in C , 13.75 % in PN and 19.12 % in PX groups of birds. Thereafter, the blood glucose levels started falling steadily and attained the fasting levels by about 120 minutes post-infusion in the C and PN groups. However, in the case of PX group, the glycemic level had fallen below the fasting level by about 60 minutes itself, whereafter it kept falling and remained at a subnormal level (-28.51 %) by about 120 minutes post-infusion.

For the IRT test, the normal fasting glycemic level prior to insulin injection was 248.20 mg/100 ml and 164.52 mg/100 ml in C and PX groups respectively. The birds of PN group had an intermediate level of 220.67 mg/100 ml. The fall in glucose level after injection of insulin lasted for 120 minutes in birds of C and PN groups whence the levels had fallen by 49.99 and 47.32 % respectively. Against this the PX group depicted a fall of 51.81 % by 90 minutes only. The subsequent elevation in the glycemic level lasted a uniform 120 minutes from the time of attainment of maximum hypoglycemia in all the three groups and thus reached the respective zero (normal) levels by 4 hrs (in C and PN groups) and 3½ hrs (PX groups).

TABLE-1: Seasonal changes in GTT in normal, sham operated and pinealectomised wild pigeons.

Time in minutes		BREEDING PERIOD Glucose levels in blood mg/100ml			NON-BREEDING PERIOD Glucose levels in blood mg/100ml		
		PN	PX	C	PN	C	PX
Zero		199.99 ± 18.63	190.00 ± 13.94	216.00 ± 0.0	206.85 ± 9.06	208.58 ± 10.56	180.96 ± 27.04
30		227.49 ± 11.69	226.33 ± 16.72	240.83 ± 10.63	249.71 ± 14.67	248.64 ± 22.52	248.84 ± 22.62
	P.diff	+ 13.75	+ 19.12	+ 11.49	+ 20.72	+ 19.20	+ 37.51
60		203.83 ± 5.59	174.99 ± 25.90	237.5 ± 17.67	256.66 ± 5.59	260.41 ± 22.18	241.38 ± 26.32
	P.diff	+ 1.92	- 7.9	+ 9.9	+ 24.08	+ 24.84	+ 33.88
90		200.00 ± 9.13	142.16 ± 8.80	220.83 ± 17.67	250.33 ± 4.77	245.82 ± 7.21	232.22 ± 17.08
	P.diff	+ 0.005	- 25.17	+ 2.23	+ 21.02	+ 17.85	+ 28.32
120		198.05 ± 9.13	135.83 ± 13.69	216.66 ± 23.56	205.41 ± 2.50	204.66 ± 2.97	218.88 ± 17.50
	P.diff	- 0.970	- 28.51	+ 0.30	- 0.696	- 1.87	+ 20.95

PN - Sham operated, C- intact controls, PX - Pinealectomised P.diff - Percentage difference from normal glucose level('0'minute)

TABLE-3: GTT and IRT induced seasonal alterations in percentage rate of glucose elevation, percentage rate of glucose clearance and percentage clearance and percentage normalisation respectively.

	Breeding Period			Non-Breeding Period		
	PN	PX	C	PN	PX	C
E	0.458	0.637	0.383	0.401	1.25	0.414
K	0.143	0.444	0.111	0.332	0.133	0.356
$\frac{E}{K}$	3.20	1.43	3.45	1.20	9.39	1.16
Ki	0.399	0.576	0.417	0.453	0.367	0.307
Ni	0.688	0.854	0.779	0.203	0.370	0.190
$\frac{Ki}{Ni}$	0.579	0.674	0.535	2.23	0.99	1.61

E - Glucose elevation rate

K - Glucose clearance rate

Ki - Insulin induced glucose clearance rate

Ni - Insulin induced glucose normalisation rate

PN - Sham pinealectomised

PX - Pinealectomised

C - Intact control.

Figs. 1 : GTT induced alterations in the glycemic levels in normal, sham operated and pinealectomised pigeons in the breeding season.

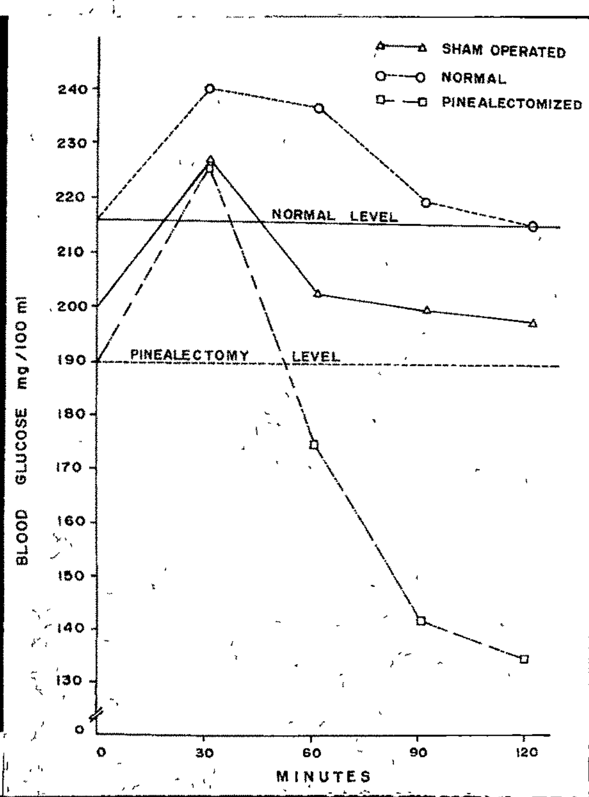

Fig 1

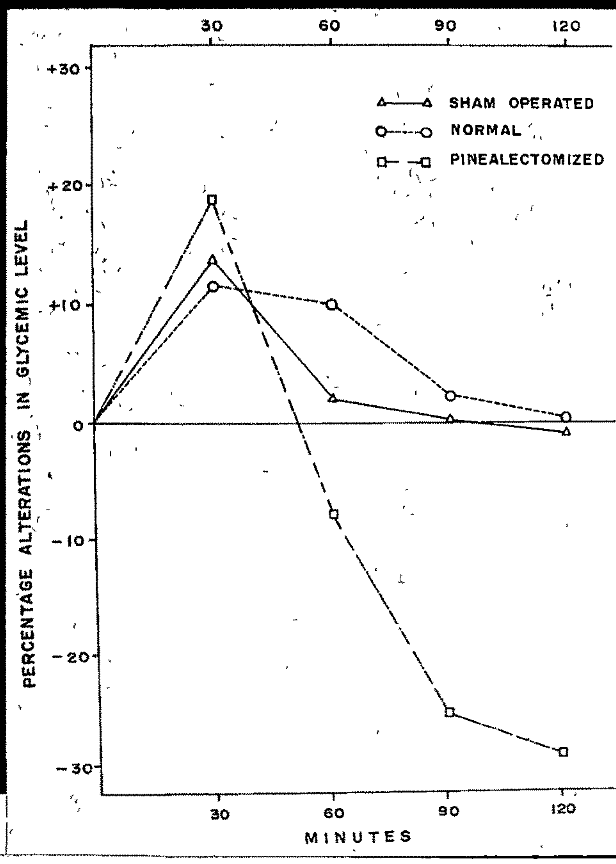

Fig 1a

Fig 1a : Percentage alterations in glycemic level after glucose loading in normal, sham operated and pinealectomised pigeons during the breeding season.

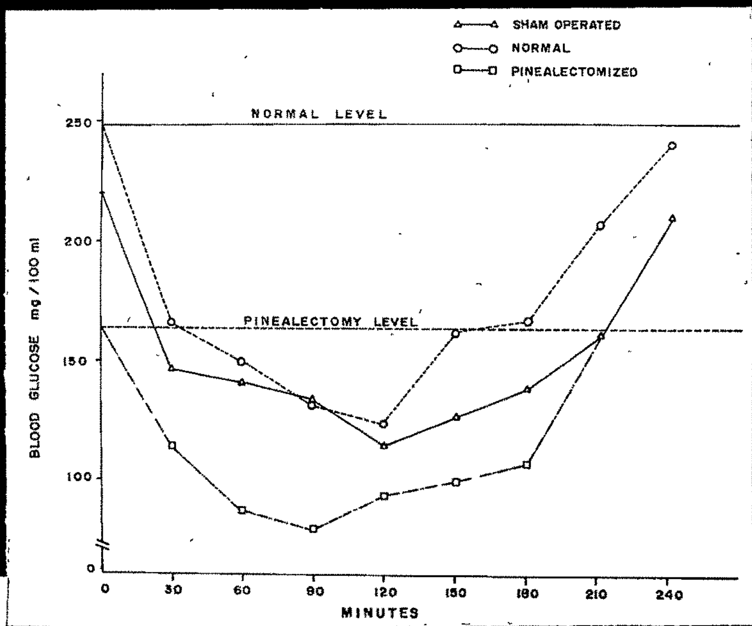

Fig 2

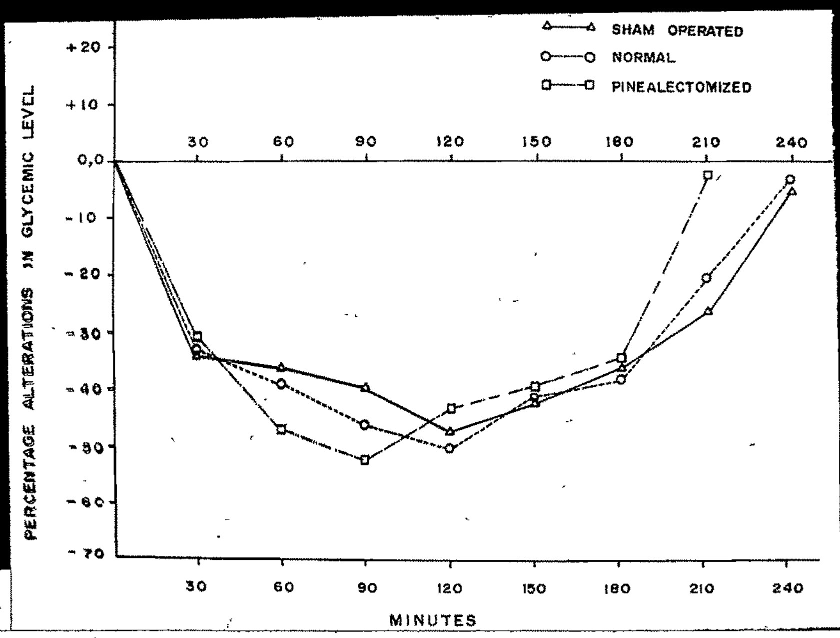

Fig 2a

Figs. 2: Alterations in the glycemic levels after insulin injection in C, PN and PX birds during the breeding season.

Fig. 2a: Percentage alterations in the glycemic levels after insulin injection in C, PN, and PX birds during the breeding season.

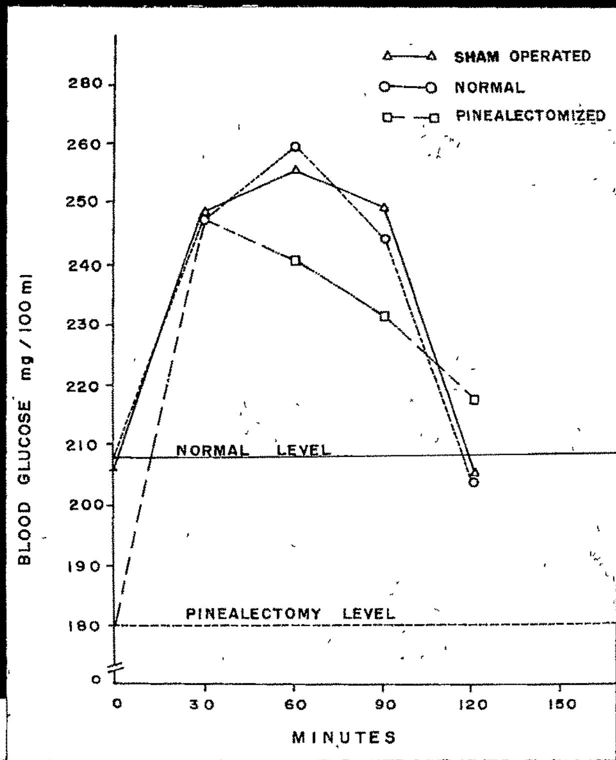

Fig 3

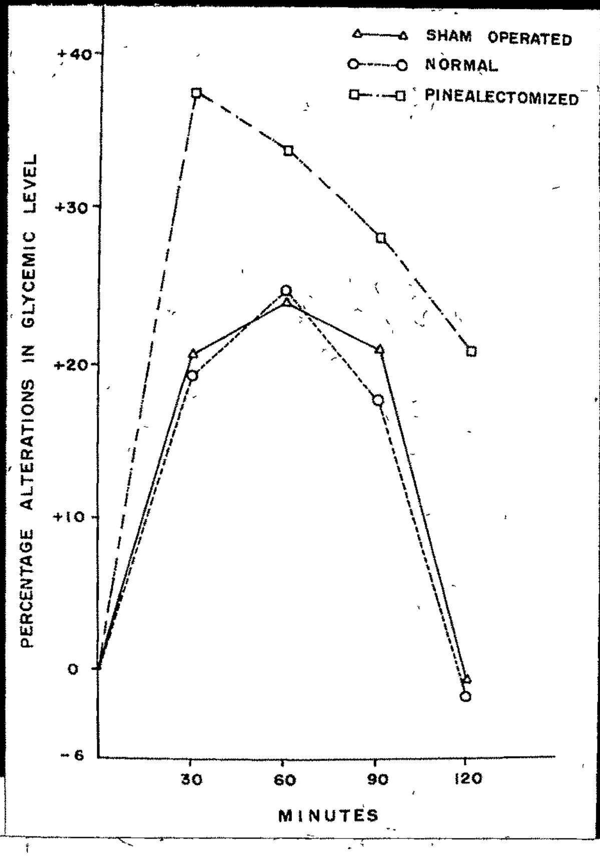

Fig 3a

Fig. 3 : GTT induced alterations in the glycemic levels of C, PN and PX birds during the non-breeding season.

Fig.3a : Percentage alterations in the glycemic levels after glucose loading in C, PN and PX birds during the non-breeding season.

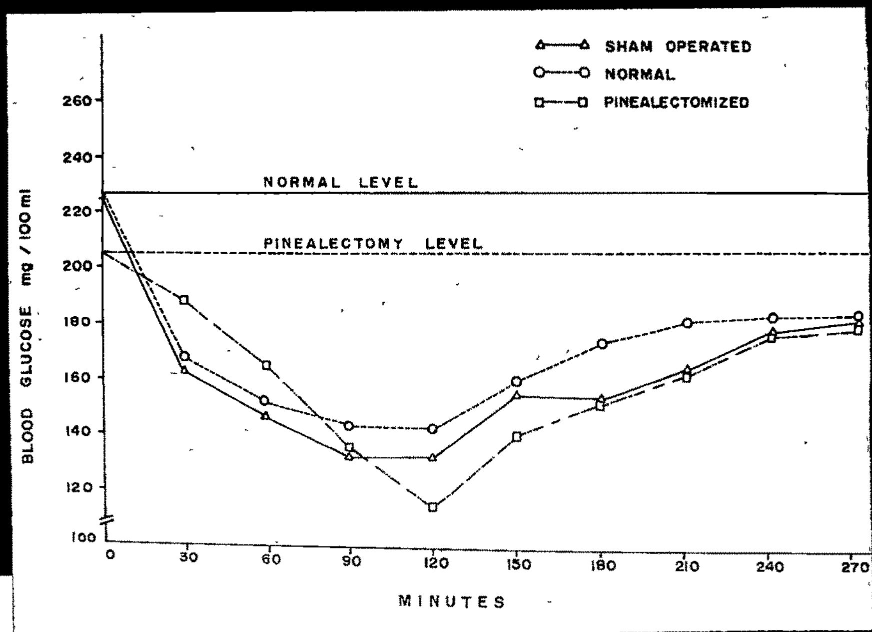

Fig 4

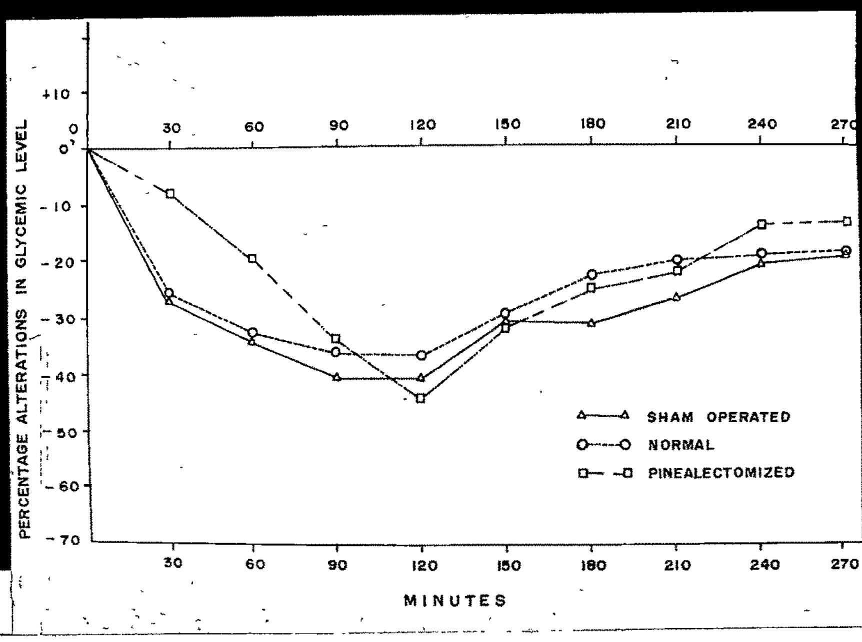

Fig 4a

Fig. 4 : Changes in the glycemic levels after insulin injection in C, PN and PX birds during the non-breeding season.

Fig.4a : Percentage alterations in the glycemic levels after insulin injection in C, PN and PX birds during the non-breeding season.

GTT AND IRT DURING THE NON-BREEDING SEASON

The fasting blood glucose level prior to glucose infusion in the three groups were 208.58 mg/100 ml (C), 206.85 mg/100 ml (PN) and 180.96 mg/100 ml (PX). After glucose infusion the glycemic level was elevated to the maximum (37.51 %) by 30 mts. in PX group, while the maximum elevation occured in C and PN groups (24.84 % and 24.08 % respectively) by 60 mts. only. Since then, whereas in the C and PN groups the glycemic levels had reached the respective fasting levels within the next 60 mts, that in the PX group at 120 minutes was still 20.95 % above normal.

The fasting glycemic level prior to insulin injection was 226.66 mg/100 ml, 224.66 mg/100 ml and 205.59 mg/100 ml in C, PN and PX groups respectively. Post-insulin injection hypoglycemia lasted 120 mts. in all the three cases whereafter the blood glucose levels started increasing gradually and even at the end of 4½ hrs. the glycemic levels were below normal by 19 to 13 % in the control and pinealectomised groups respectively.

The levels of blood glucose in terms of time intervals and the percentage changes under both GTT and IRT are shown in tables 1 and 2 and figures 1,1a and 2,2a. To have a better understanding, the per minute % rate of glucose elevation (E) and the per minute % clearence rate (K) under GTT, and the insulin induced percentage clearance rate (Ei) and the post-insulin % glucose normalisation rate (Ni) are also calculated and given in table-3. The table also includes the $\frac{E}{K}$ ratio as well as $\frac{Ki}{Ni}$ ratio.

DISCUSSION

The glucose tolerance test usually provides clue to the functional status of pancreas, specifically β cell functioning. Decreased glucose tolerance is usually indicative of reduced functional ability of β cells and increased glucose tolerance would therefore be indicative of the reversed functional status of pancreas. Whereas GTT can give an idea of the β cell functioning, alterations induced in the glycemic level in response to insulin injection can provide informative idea about the degree of insulin sensitivity (positive or negative) in the animal. The present study conducted as a follow up of the previous studies on seasonal alterations in carbohydrate metabolism shown by intact and pinealectomised wild pigeons (Patel,1982), has again provided convincibility to the concept of seasonal alterations in both normal and pineal ablated birds. Season specific adaptive modulations in the glycemic level as well as glycogen stores of the body mediated by the differential rates of secretion of pancreatic hormones or by their altered sensitivity can easily be presumed to occur in intact wild pigeons from the results accrued herein. From the tables 1,2 and figures 1,1a,2,2a it becomes evident that subsequent to glucose loading while there is 12 % elevation in glucose level in 30 minutes in the breeding period, there is a 25 % increase in 60 minutes in the non-breeding period. However the glycemic level returns to the normal levels by 90 minutes and 60 minutes respectively in the two seasons. Apparently these points indicate a reduced insulin sensitivity/insulin release in the breeding season and better insulin sensitivity/insulin release in the non-breeding season. Glucose a known inducer of insulin release in mammals is reported to be ineffective as a promoter of insulin release from avian pancreas at levels less than 500 mg % (Hazelwood, 1977). It is nevertheless imperative that under a glucose load the glucose clearance has to be mediated by insulin and the time taken for the same can offer a good index to the insulin status of the organism. The glucose elevation and glucose clearance values (E and K) and the E/K ratio of unity during the non-breeding season suggests better insulin action. The corresponding reduced K values and higher E/K ratio (3.45) are indicative of the comparatively minimised insulin action during the breeding season. These interpretations are well borne out by the results of insulin response test. The IRT conducted during the two seasons have depicted higher Ni value (glucose normalisation value) in the breeding season and reduced Ni value in the non-breeding season with a more or less similar Ki value leading to a less than half Ki/Ni ratio during the breeding season. Obviously increasing insulin resistance to glucose elevation during the non-breeding season and decreased insulin resistance during the breeding season are inferable.

Pinealectomy induced season specific alterations in carbohydrate metabolism have been documented for wild pigeons by a previous doctoral work from this laboratory (Patel, 1982). Based on the observations thereat, an anti-insulinic role of pineal was inferred especially in the breeding season. The results of the present study on GTT and IRT of PX birds are very much distinct from those of the control birds. The hyper-glycemic condition after glucose loading in PX birds reached its maximum of 38 % and 30 % in the non-breeding and breeding periods respectively by 30 minutes itself. Though the glycemic level started falling thereafter, it was high by 21 % even at 120 minutes in the non-breeding phase. In contrast during the breeding period, within 30 minutes after the attainment of peak glycemic condition, the level had fallen below normal with the result it was subnormal by 7.9 %. This hypoglycemic trend persisted thereafter and at 120 minutes the level was 29 % below normal. The E and K values for the non-breeding period were three times more and three times less than the control values respectively, while for the breeding period both were equally higher than the controls; with the result the E/K ratio for the non-breeding period was 9 times that of the control ratio and that for the breeding period was less than half of the control ratio. In terms of IRT, the maximal hypoglycemic condition (44 %) in the non-breeding period was attained by 120 minutes, while the maximal hypoglycemic condition (52 %) in the breeding period was attained by 90 minutes only. However the glucose normalisation process was better in the breeding period as near normal glycemic level was attained by 210 minutes (i.e. within 120 minutes) as compared to the 13 % hypoglycemic level still persisting at 270 minutes in the non-breeding period. The Ki/Ni ratio of 0.99 obtained for the non-breeding period was lower than that of the controls and the same ratio of 0.674 obtained for the breeding period was slightly more than that of the controls. These points of observations indicate some sort of insulin insensitivity or inability of insulin release in response to a glucose load in PX birds during the breeding season and better insulin sensitivity or ability of glucose induced insulin release in the non-breeding season. It becomes obvious from the present studies that the pineal in tropical wild pigeons has an anti-insulinic role especially during the breeding season and such an influence is very much lacking during the non-breeding season. The report of Baily _et al._ (1974) that melatonin can suppress glucose induced insulin secretion in rat and mouse is interesting from the present point of view. Other evidences in favour are the reports of Benson _et al._ (1971) in rats and Burns (1973) in monkeys of hyperglycemia following blinding and melatonin injection respectively. Again, Murlidhar _et al._ (1982) have reported reduced glucose tolerance following melatonin injection in rabbits. All these tend to corroborate the present concept of pinealectomy induced increased glucose tolerance in tropical wild pigeons. The sluggish rate of glucose elevation observed in the non-breeding period in both the control and PX birds indicates poor glucagon response in this season and may have some relevance with the reported insulin stimulated somatostatin secretion and its inhibition of glucagon secretion in chicken (Richard and Weir, 1979).

In conclusion it could be stated that under increasing blood glucose concentration, insulin release is reduced during the breeding period probably to permit maintenance of elevated glucose levels and even glycogenolysis which may both be essential for metabolic adaptation associated with breeding. Converse seems to be true for the non-breeding phase. In this context the increased adrenocortical activity during breeding (Ramachandran et al., 1984b) and the report of Plager and Matsui (1966) of an anti-insulinic action of cortisol may be relevant. Pinealectomy seems to negate these adaptations characteristic of intact birds thereby inducing higher insulin action during breeding and reduced insulin action during non-breeding. Again, the pinealectomy induced suppression of adrenocortical activity in the breeding period is noteworthy (Ramachandran et al., 1984b).