CHAPTER - X

SEASONAL ALTERATIONS IN GLUCAGON AND ADRENALIN RESPONSES IN NORMAL AND PINEALECTOMISED WILD PIGEONS, *COLUMBA LIVIA*

Carbohydrate reserves of the body represented by tissue glycogen contents and circulating blood glucose serve as efficient and immediate energy sources for the many seasonal physiological events. One of the most important seasonal physiological activity requiring increased energy expenditure is that of reproduction. In order to meet the requisite energy demands, alterations in carbohydrate homeostasis is brought about. Such adaptive alterations in carbohydrate homeostasis is usually under the purview of the neuroendocrine system. The principal hormones associated with carbohydrate metabolism are the pancreatic hormones-insulin and glucagon, the pituitary hormonessomatotropin and the adrenal hormone-corticosterone or corticosterol. Of these, all except insulin have hyperglycemic action with glucagon being glycogenolytic in nature and corticosteroid gluconeogenic. Apart from the cortical glucocorticosteroid, the adrenal medullary hormone adrenalin also exerts influence on carbohydrate metabolism. Like glucagon, adrenalin also has glycogenolytic action. Previous studies on intact and pinealectomised wild pigeons conducted on a seasonal basis *vis a vis* breeding, had shown alterations in carbohydrate metabolism (Patel, 1982). Though utilization of hepatic glycogen store was an essential feature associated with breeding in intact birds, pinealectomy was noted to induce both hypoglycemia as well as depletion in hepatic glycogen store. These were taken to indicate a possible anti-insulinic action of pineal during the breeding season in wild pigeons. Atleast a few workers have attempted to study the pineal-pancreas axis either by injections of melatonin or by pinealectomy and their results are suggestive of some involvement of pineal in carbohydrate metabolism (Mckeowm <u>et al</u>., 1975; Delahunty <u>et al</u>., 1978; Mihail and Giurgea, 1979). Differing titres of the hormones together with their altered sensitivity and synergism or antagonism between them are factors which usually determine the metabolic profile at any time. Such factors could easily be purported to be involved in the normal seasonal alterations in the carbohydrate reserves of the body as well as pinealectomy induced disturbances thereat. In this respect the responses to injections of glucagon and adrenalin in terms of glycemic levels can give some idea about the differential seasonal sensitivity towards these hormones as well as the extent of their action. Both glucagon and adrenalin have been used widely to test their glycogenolytic and hypoglycemic actions in different groups of vertebrates (Marques, 1967; Farrar and Frye, 1977; Daniel and Norman, 1978; Keller and Alina, 1979; Moura <u>et al</u>., 1982). By far glucagon is responsible for hepatic glygenolysis and hyperglycemia by activating the adenylate cyclase-cAMP system in the liver cells, while adrenalin though principally known to induce muscle glycogenolysis is also capable of bringing about hepatic glycogenolysis and hyperglycemia by way of the Ca^{++}-calmodulin regulatory mechanisms (Park, 1981). Since the breeding and non-breeding seasons were observed to induce altered profile of carbohydrate metabolism in both the intact and pinealectomised pigeons (Patel, 1982), the glycemic responses to single injection of glucagon and adrenalin in normal, sham operated and pinealectomised wild pigeons are studied currently.

MATERIAL AND METHODS

Adult feral blue rock pigeons procured from the local animal dealer and maintained on a diet of grains and water *ad libitum* were used for the present experiment. Birds of both the sexes were divided into three groups of six each during both the breeding and non-breeding seasons. The three groups represent the intact control (C), sham pinealectomised control (PN) and pinealectomised (PX). Pinealectomy was performed as described previously and the birds were taken for glucagon response and adrenal response tests 30 days post-surgery along with the corresponding control birds. The control birds were also maintained along with the pinealectomised birds under similar conditions (i.e. natural photoperiod, grains as diet and water *ad libitum*).

GLUCAGON RESPONSE TEST (GRT)

GRT was assessed by injecting a single dose of glucagon obtained from Sigma Chemicals (0.1 mg in 0.1 ml of redistilled water I.V. i.e. 333 μg/kg body weight) to all the three groups of birds and the blood glucose levels were measured from samples drawn from the wing vein at regular intervals of 30, 60, 90,120, 150, 180, 210, 240 and 270 minutes after the injection. Prior to glucagon injection the birds were fasted over-night and the fasting blood glucose level (zero level) was estimated.

ADRENALIN RESPONSE TEST (ART)

ART was studied in terms of glycemic levels in all the three groups of birds. A single injection of adrenalin (0.25 ml containing 0.45 mg I.V. i.e. 1.5 mg/kg body weight) was given to over-night fasted birds and the glucose level estimated at zero hour (prior to adrenalin injection) and 30, 60, 90, 120, 150, 180, 210, 240 and 270 minutes post-adrenalin injection.

RESULTS

The glycemic level after glucagon and adrenalin injections along with the percentage changes are represented in the tables 1 and 2 and figures 1,1a, 2 and 2a. Tables 1 and 2 depict the glucagon and adrenalin induced per minute percentage glucose elevation rate (Eg and Ea respectively), per minute percentage glucose clearance rate (Kg and Ka) and the $\frac{Eg}{Kg}$ and $\frac{Ea}{Ka}$ ratios which are calculated for the sake of easy understanding and interpretation (Table 3).

SEASONAL ALTERATIONS IN GRT

The glucagon induced hyperglycemia was found to be prolonged in both control and experimental animals and reached a maximum by 150 minutes in the breeding season. Comparatively higher percentage of glucose elevation was seen in the PN and PX birds than that observed in (C) birds. In the non-breeding season too the peak glycemic level was attained by 150 minutes post-injection in the controls while it occured by 120 minutes in the experimental birds. Between the two seasons, higher percentage of glucose elevation was characteristic of the breeding season. While the maximum percentage glucose elevation was of the order of 56.6 % and 61.2 % in C and PN birds and 83.7 % in PX birds in the non-breeding season, the corresponding glucose levels for the breeding season were 78 %, 117.6 % and 101.2 % . In either season normo-glycemia was not attained even by 300 minutes (5 hrs) post-injection. Between the three groups of animals, the Eg and Kg values for either of the two seasons were not much different. Between the two seasons the Eg values were lower in the non-breeding season and the Kg values higher with the result the Eg/Kg ratio was less during the non-breeding period and high during the breeding period.

TABLE - 3 : Seasonal alterations in the percentage rate of glucose elevation, percentage rate of glucose clearance and the ratio of elevation to clearance.

	Breeding Period			Non-Breeding Period		
	PN	PX	C	PN	PX	C
Ea	0.638	0.508	0.674	0.394	0.696	0.329
Ka	0.231	0.202	0.248	0.067	0.043	0.063
$\frac{Ea}{Ka}$	2.76	2.81	2.71	5.88	16.18	5.22
Eg	1.17	1.12	0.824	0.510	0.698	0.377
Kg	0.095	0.154	0.097	0.135	0.246	0.166
$\frac{Eg}{Kg}$	12.31	7.27	8.49	3.77	2.83	2.27

Ea - Adrenaline induced glucose elevation rate

Ka - Adrenaline induced glucose clearance rate

Eg - Glucagon induced glucose elevation rate

Kg - Glucagon induced glucose clearance rate

PN - Sham pinealectomised

PX - Pinealectomised

C - Intact controls.

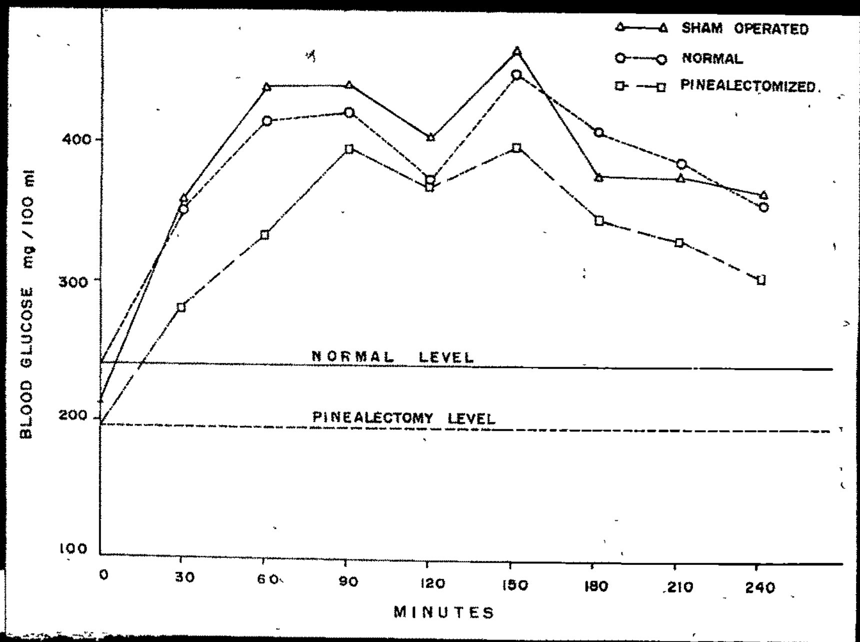

Fig 1

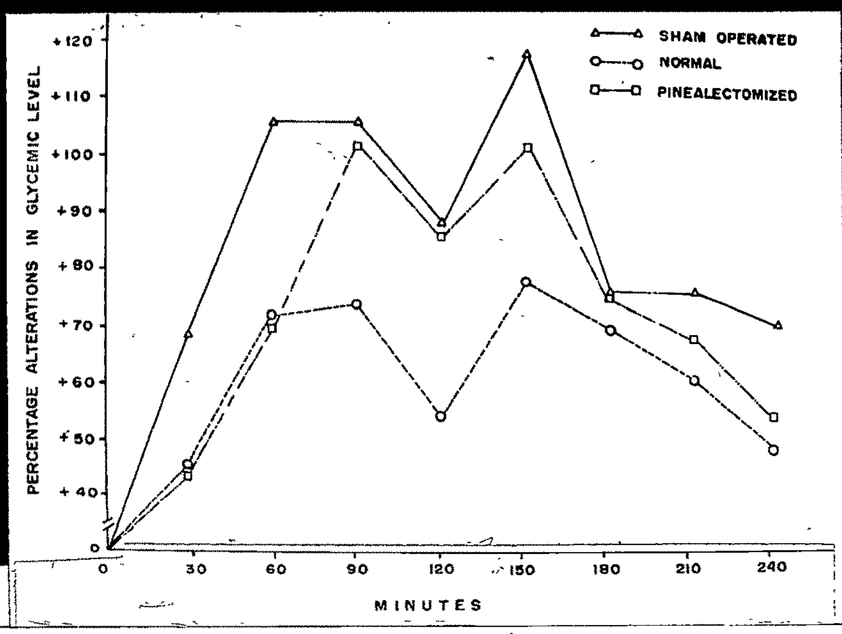

Fig 1a

Fig 1 : Alterations in the glycemic levels after glucagon injection in C,PN and PX birds during the breeding season.

Fig 1a: Percentage alterations in the glycemic levels after glucagon injection in C,PN and PX birds during the breeding season.

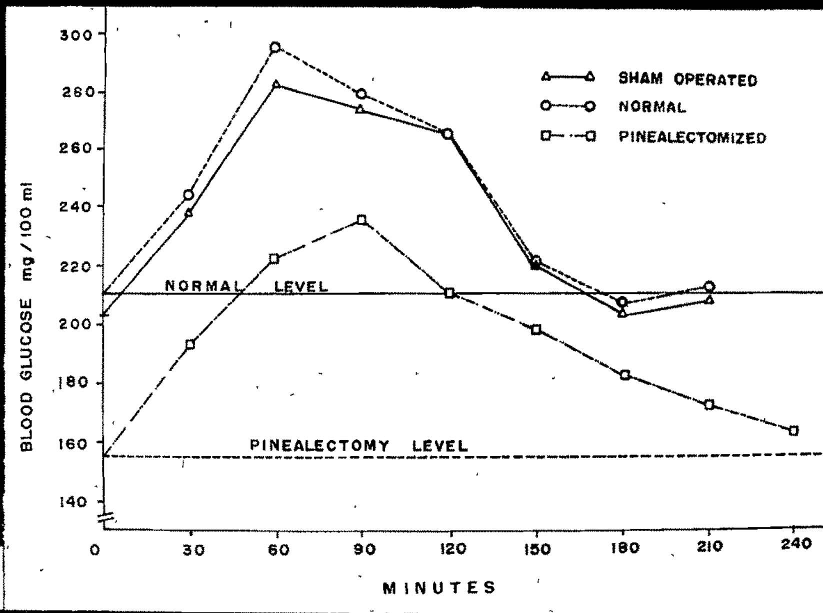

Fig 2

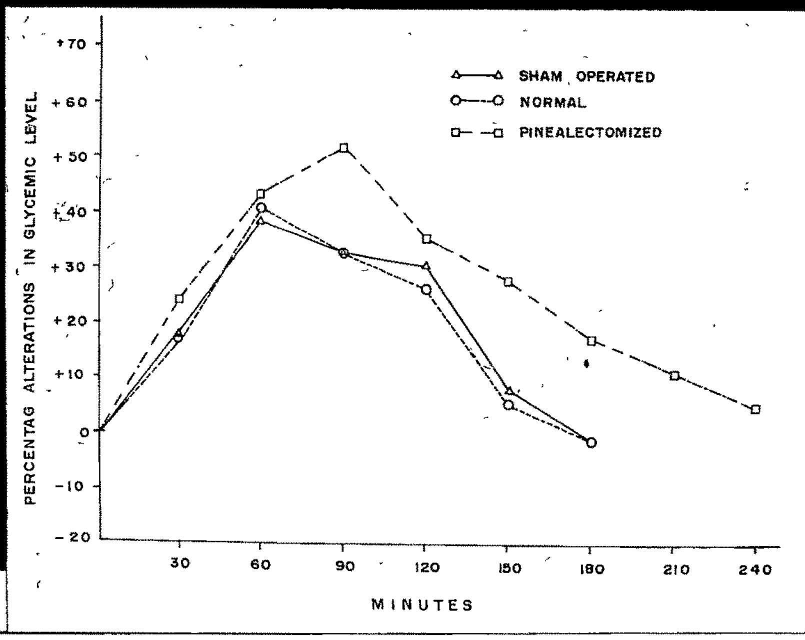

Fig 2a

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

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

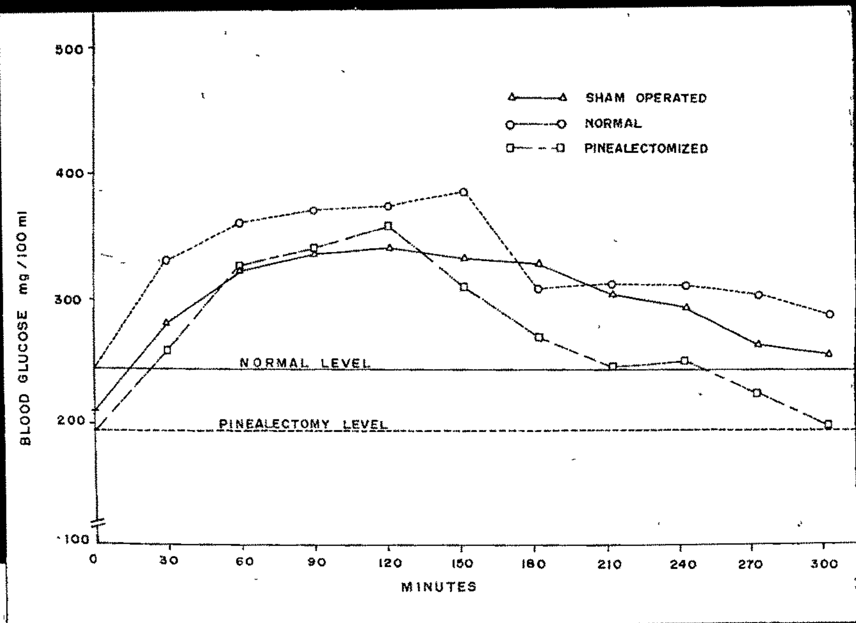

Fig 3

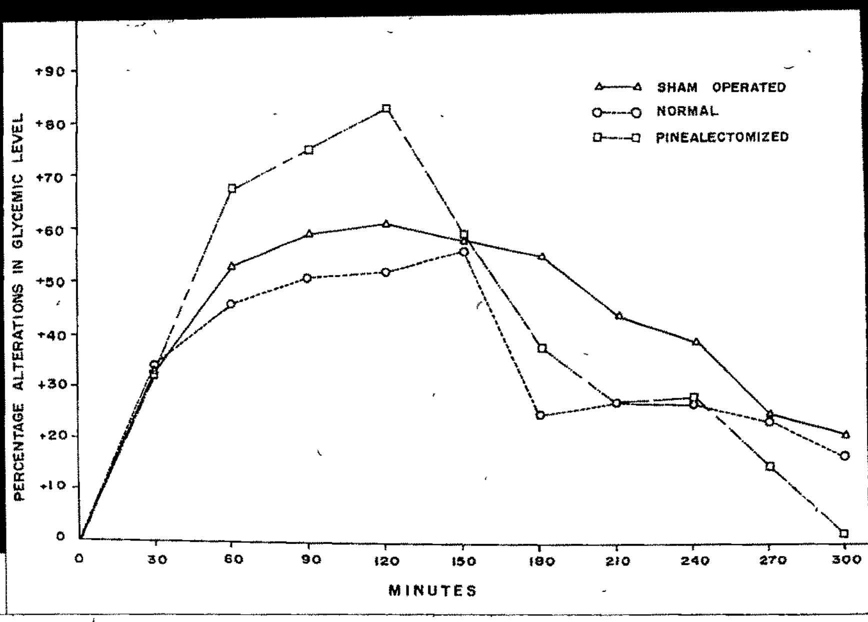

Fig 3a

Fig. 3 : Alterations in the glycemic levels after glucagon injection in C, PN and PX birds during the non-breeding season.

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

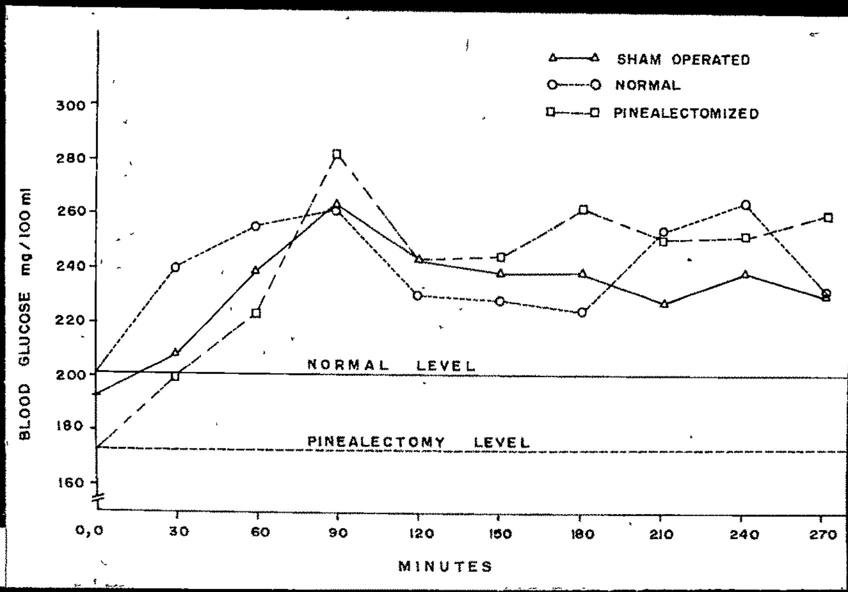

Fig 4

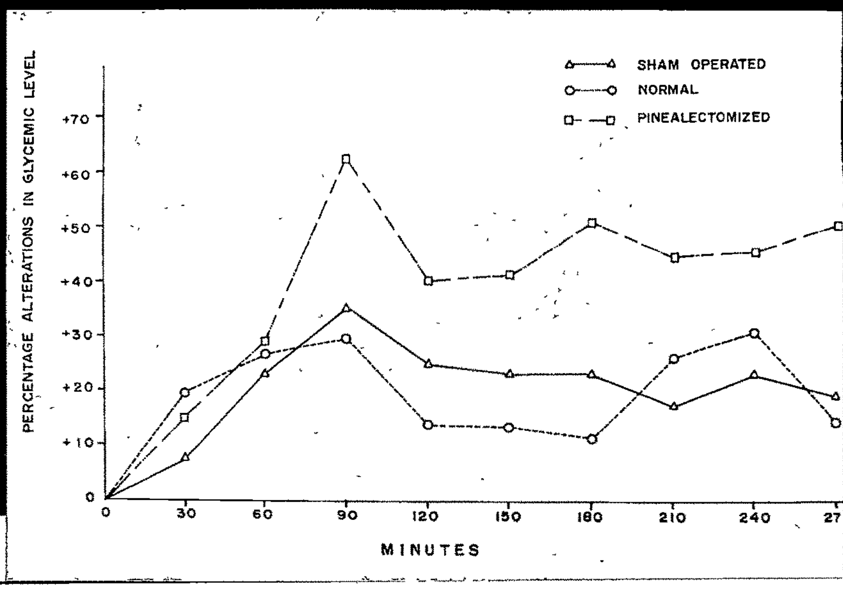

Fig 4a

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

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

SEASONAL ALTERATIONS IN ADRENALIN RESPONSE TEST (ART)

The glycemic response to adrenalin injection was varied in the two seasons. In the intact birds the maximum glycemic level was reached by 60 minutes (40 %) after injection in the breeding season while the same was achieved only by 90 minutes (30 %) in the non-breeding season. Whereas normo-glycemia was attained by 3 hours in the breeding season, the glycemic level in the non-breeding season was 15 % above normal even at 4½ hours after injection. Pinealectomised birds too have shown a more or less similar response with maximum glycemic level being attained by 90 minutes in both the seasons. The percentage increase at this stage was 51 % in the breeding season and 63 % in the non-breeding season. At the end of 4 hours after injection the glycemic level was only 5 % above normal in the breeding season while it was 50 % above normal even at 4½ hours in the non-breeding season.

DISCUSSION

The responses to glucagon and adrenalin in terms of temporal glycemic level would greatly depend on the quantitative and qualitative balance of interactions of various other hormones. The balance of interactions is bound to be different on a seasonal basis due to the alterations in the activity of various endocrine glands. Effects of glucagon and adrenalin on glycemic levels and tissue glycogen contents though have been studied in all classes of vertebrates, very few of them are conducted on a seasonal basis. In the present study the glycemic responses to single injections of these two hormones have been assessed during both breeding and non-breeding periods of tropical wild pigeons. An additional variant in the study is presence or absence of pineal. Both glucagon and adrenalin are hyperglycemic hormones which bring about hepatic glycogenolysis. However, it is established that whereas glucagon induces glycogen breakdown by activating the adenylate cyclase-cAMP system, adrenalin activates glycogenolysis by an α receptor system activating the Ca^{++}-calmodulin system (Park, 1981). The present study principally conducted to understand the pinealectomy induced seasonal alterations if any on the influence of these hormones in the regulation of carbohydrate metabolism has not shown very significant changes unlike that had been seen previously for insulin (Chapter-9).

From the tables 1 and 2 and Figures 1,1a,2 and 2a it is evident that the glucagon response of intact birds is greater during the breeding season than during the non-breeding season as there was a 74 % increase in glycemic level within 90 minutes in the former season as opposed to that of 57 % in 150 minutes in the latter season. The same holds good for adrenalin response too with a 40 % increase in 60 minutes in the breeding season against the 30 % increase in 90 minutes in the non-breeding season. The normalisation process subsequent to the hyperglycemia induced by both the hormones was better in the non-breeding period than in the breeding season which indicates the reduced insulin action during the breeding season in intact pigeons. Higher elevation rate during the breeding phase as opposed to the higher clearance rate during the non-breeding phase are also suggestive of the same. This inference is confirmatory to the earlier findings (Patel, 1982; Chapter-9). The seasonal variations in glucagon and adrenalin responses noted currently for pigeons in terms of breeding activities have no parallel in other birds or even in other vertebrates. The only reports of seasonal bias are those of Marques (1967) showing better hyperglycemic response to injections of glucagon during winter than summer in turtles and of Farrar and Frye (1977) showing better hyperglycemic response to glucagon in summer and fall than winter and the same for adrenalin in fall than in summer and winter in Rana pipiens. Though the above studies do indicate some seasonal differences in response, they are not however related in terms of breeding phases.

The above reported seasonal alterations characteristic of intact pigeons appear to be offset by pinealectomy as could be inferred from the responses shown by PX birds. With reference to glucagon response, PX birds have shown increased sensitivity during both the seasons which on a comparative basis was more pronounced in the breeding season. Compared to the corresponding controls, PX induced 1.5 times more percentage glucose elevation. This is obvious from the higher Eg values; but as the Kg values are also greater than that for controls the Eg/Kg ratio appears identical to those of intact birds. Evidently PX does not alter the glucagon response quantitatively. Adrenalin action in the PX condition seems to be more pronounced during the non-breeding season than during the breeding season. Increased elevation rate coupled with decreased clearence rate and the resultant significantly high Ea/Ka ratio lend credence to the same. The normalisation process after the hyperglycemic response to glucagon is reduced during the non-breeding season in both intact and PX pigeons. Incidentally insulin response was better during this season (Chapter-9). The known adrenalin antagonism to insulin could be the reason for the reduced clearance rate observed during the non-breeding season. However, the present observations also demonstrate a better clearance rate in the PX birds during the breeding season where insulin action was considered to be very high (Chapter-9). It is likely that pinealectomy induces such a great insulin release/insulin action in response to hyperglycemia that the dose of adrenalin injected in the present study is not sufficient to exert a complete insulin antagonistic action. Another point of discordance that could be noted is the attainment of a complete normo-glycemic level subsequent to adrenalin induced hyperglycemia within only 120 minutes in the intact animals during the breeding season. A possible ability of higher titres of adrenalin to neutralize the anti-insulinic effect of pineal in this light needs to be ascertained. Harri and Hedestam (1972) have shown calorigenic effect of adrenalin by increased oxygen consumption in Rana Temporaria in a season specific fashion. Based on this observation, the likely ability of adrenalin to bring about increased blood glucose utilisation in the intact pigeons during the breeding season may also be relevant. One very significant observation of the present study is the glycemic response to adrenalin shown by all the three groups of birds in the non-breeding season. Whereas the C and PN groups of birds tended to show a biphasic increase in glycemic level; an initial increase in the first 90 minutes and a later increase between 200-240 minutes, the PX birds showed a triphasic response with the first elevation in the first 90 minutes, the second elevation between 150-180 minutes and the third increase between 240-270 minutes (See tables 1 to 3, figures 1,1a,2 and 2a). This pattern of glycemic response is once again suggestive of the increasing resistance to normalisation of the glycemic level occuring during the non-breeding season. Such a resistance occuring to a great extent in PX birds is indicated by the high Ea value, low Ka value and the resultant very high Ea/Ka ratio.

It may be said from the present study that the glucagon and adrenalin sensitivities are more during the breeding season than during the non-breeding season. Pinealectomy does not seem to induce much alteration in glucagon response while it does have some influence on adrenalin responses which is more season specific. These alterations are seemingly related to the seasonal actions of pineal and need to be more carefully investigated to get a proper perspective of pineal-pancreas-adrenal axis.