Chapter III

CIRCANNUAL VARIATIONS IN CARBOHYDRATE METABOLISM IN RELATION TO TESTICULAR CYCLICITY AND THE INFLUENCE OF PINEALECTOMY OR ALTERED ADRENOCORTICAL ACTIVITY.

Reproductive activities involve heavy energy expenditure and, all animals divert sizeable amount of the energy provisions for meeting the exigencies of breeding. This becomes more significant in seasonal breeders as their annual budgetary provisions have to be reallocated in a particular season to meet the energy requirements associated with reproductive activities. Obviously, seasonal breeders could be expected to show over the year variations in energy metabolism. Though the energy metabolism of domestic and poultry birds has been an object of study for many years, this aspect has not received attention in the feral species. A proper understanding of proportionate seasonal budgetary allocation can be meaningful only when the circannual variations on the whole is known. Moreover, alterations occurring due to experimental manipulations in any season can become more coherent and meaningful only when viewed in relation to factors and exigencies operating on a circannual basis. Carbohydrates occupy a primary status in energy metabolism. Importance of carbohydrates in the energy metabolism of Indian feral pigeons in relation to testicular

cyclicity has been shown by the changes in the levels of tissue glycogen and blood glucose in the various reproductive phases (Ayyar,1987; Patel et al.,1988; Patel, 1993). Besides, pinealectomy (PX) and induced adrenocortical insufficiency have been shown to induce testicular regression in the breeding phase and also concurrently alter plasma glucose level and tissue glycogen content (Ayyar, 1987; Ramachandran et al.,1987; Ramachandran and Patel,1988; Patel et al, 1988). Since the earlier studies had revealed a parallel adrenal-testes relationship, alterations in carbohydrate metabolism induced by the administration of either ACTH or CORT in the non-breeding season were also evaluated (Ayyar, 1987). Though the above observations suggested definite alterations in carbohydrate metabolism, the exact relationship between carbohydrate metabolism and gonadal cyclicity needed ascertainment. The rationale behind the present study in this context was to work out the normal circannual variations in carbohydrate metabolism (by monthly evaluation) and to see, whether the alterations induced by experimental manipulations (involving pineal and adrenal) affecting testicular activity, are related to the functional status of the testes or are independent. To this end, alterations in carbohydrate metabolism, in terms of PX or adreno-cortical suppression in the breeding season and CORT administration to PX birds in the breeding or regression phases and, CORT administration to intact birds in the

regression or quiescent phases, have been assessed.

MATERIALS AND METHODS.

Procurement and maintenance of pigeons.

Between December 1990-December 1992 birds were procured every month from a local animal dealer and were housed in a well ventillated aviary with food and water ad libitum. The annual testicular cycle of feral pigeons can be sub-divided into five distinct phases : (1) Breeding phase (March-May) (2) Regression phase (May-Aug.). (3) Quiescent phase (September-December). (4)Pre-recrudesscent phase (December-January) and (5) Recrudescent phase (January-February). During each reproductive phase, the reproductive status of the birds was confirmed by examining the testicular condition with the help of an endoscope.

Experimental setups.

1. Circannual studies.

Every month (Jan.-Dec.) 5-6 pigeons were used to study the annual cyclicity in relation to carbohydrate metabolism.

2. Experimental manipulations.

In the breeding phase (April), 30 male pigeons were randomly selected and divided into five groups of six each.

Group I (Control). These birds were given daily (ip) injections of 0.9% saline.

Group II (Dexamethasone : DXM). These birds were given daily (ip) injections of 40 µg DXM in 0.1 ml saline.

Group III(PX). These birds were subjected to surgical pinealectomy.

Group IV (PX + CORT). Pinealectomised birds were given daily (ip) injections of 2 µg CORT in 0.1 ml vehicle at 09.00h.

Group V (PXV). Pinealectomised birds were given daily (ip) injections of 0.1 ml vehicle.

Since none of the parameters studied presently showed any variation between saline treated control and vehicle treated ones, data of only saline treated controls are presented throughout.

In the regression phase (May), 30 male pigeons were randomly selected and divided into four groups of 6 each.

Group I (Control). These birds were given daily (ip) injections of saline.

Group II (CORT). These birds were given daily (ip) injections of 2 µg CORT at 09.00h.

Group III (PX). These birds were subjected to pinealectomy.

Group IV (PX + CORT).Pinealectomised birds were given daily (ip) injections of 2 µg CORT.

In the quiescent phase (Oct), 12 male pigeons were randomly divided into two groups of 6 each.

Group I (Control). These birds were given daily (ip) injections of saline.

Group II (CORT). These birds were given daily (ip) injections of 2 µg CORT at 09.00h.

All the treatment schedules in all the seasons were for 30

days.

Parameters and Methods of evaluations.

Blood glucose. Prior to decapitation of pigeons, 0.1 ml of blood was drawn from the brachial vein by a needle prick. Blood glucose level was estimated by the method of Winckers and Jacobs (1971). The glucose cencentration was expressed as mg/dl.

Tissue glycogen content.

The tissue (liver, muscle and testis) glycogen content was estimated by employing the method of Seifter et al.(1950). The colour intensity was read colorimetrically at 620 nm and the glycogen content was expressed as mg/100mg tissue weight.

Hepatic Glucose-6-Phosphatase (G-6-P'ase/E.C.3.1.3.9).

The enzyme activity was assayed by the method of Harper (1960). Inorganic phosphate released was estimated as per the method of Fiske and Subbraw (1925) and the colour intensity was read at 660 nm on a Klett Summerson colorimeter. Enzyme activity was expressed as µg phosphate released/mg protein/15min.

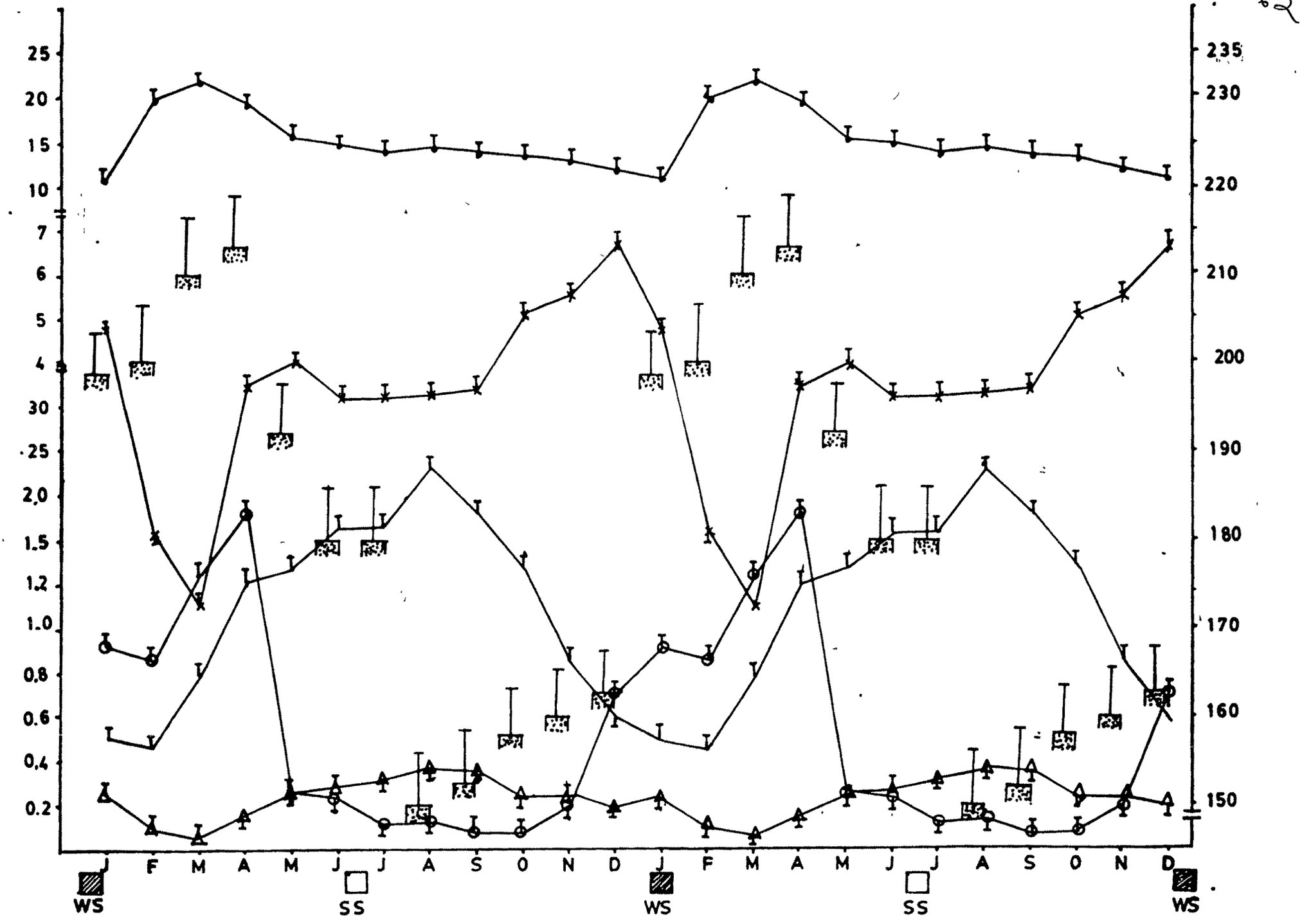
Hepatic Phosphorylase (E.C.2.4.1.1.)

Total phosphorylase activity was assayed by the method of Cahill et al. (1957). The inorganic phosphate released was measured by the method of Fiske and Subbaraw (1925). Enzyme activity was expressed as µg phosphate released/ mg protein/15 min.

RESULTS.

A. Changes in carbohydrate metabolism during annual gonadal cyclicity.

The variations in carbohydrate metabolism in relation to testicular cyclicity is shown in Fig.3A . In the months of February-April (breeding season), when the testes are active, the blood glucose level was maximum and the tissue glycogen contents at their lowest. From mid-April till December, there was a gradual decline in the blood glucose level and a steady increase in the tissue glycogen content, corresponding to testicular regression (May-Aug.) and quiescence (Sept.-Dec.). However, the glycogen content of muscle and testis reached a peak level in August-September and the hepatic glycogen content in December. The hepatic phosphorylase was lowest in January and increased to a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maximum in February-March, whereafter it started declining gradually and steadily from April onwards till a lowest level was reached in January. Changes in G-6-P'ase activity corresponded to the changes in the blood glucose level with a maximum level in the month of April and a minimum level in the month of September-October. Thereafter there was steady increase till the maximum level was reached in April.

B. Changes in carbohydrate metabolism during experimental manipulation of adrenocortical function in intact and PX pigeons.

Blood glucose and tissue glycogen content.

The blood glucose level which was higher during the breeding phase decreased significantly under induced adrenocortical insufficiency. Pinealectomy also decreased the blood glucose level in both breeding and regression phases. Corticosterone given to PX birds further decreased the blood glucose level in both the phases. However, CORT given to intact birds during the regression and quiescent phases increased the blood glucose level significantly. Tissue glycogen content was increased in birds rendered hypocorticalic during the breeding phase. There was a general tendency for decreased tissue glycogen content after corticosterone treatment to PX birds in the breeding as well as regression phases. However,

Treatments	Blood glucose (mg/dl)	Tissue glycogen (mg/100mg tissue			Hepatic Phosphorylase (µg PO_4 released /mg Protein/15 min	Hepatic G-6-P'ase (µ moles PO_4 released/mg Protein/15 min)
		Liver	Muscle	Testis		
Control	238.38 ± 7.19	3.50 ± 0.06	1.21 ± 0.09	0.16 ± 0.03	19.17 ± 0.81	1.78 ± 0.09
DXM	213.13* ± 4.07	5.49* ± 0.31	2.68* ± 0.16	0.28* ± 0.04	28.01* ± 0.44	2.18 ± 0.16
PX	197.74 ± 6.44	1.96 ± 0.27	0.96 ± 0.04	0.08 ± 0.004	18.00 ± 0.51	1.51 ± 0.09
PX+CORT	130.11* ± 7.04	0.90* ± 0.05	0.95* ± 0.05	0.01* ± 0.002	29.86* ± 0.99	2.71 ± 0.12

Table IIIa. Alteration in blood glucose, tissue glycogen content and activity levels of hepatic phosphorylase and G-6-P'ase of normal and PX pigeons subjected to functional manipulations of adrenals in the breeding phase.
(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

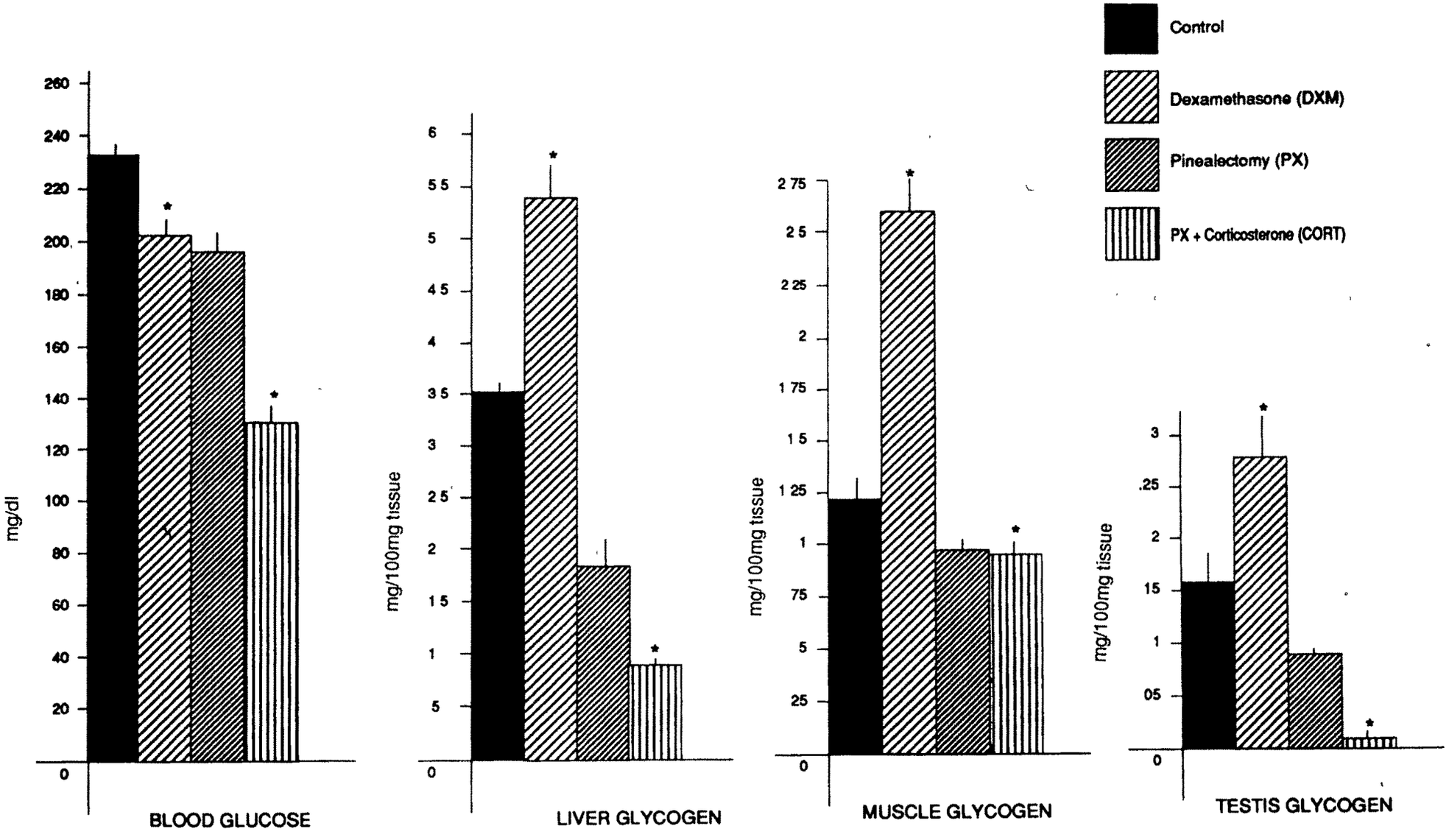

Fig. : 3 A Alterations in blood glucose and tissue glycogen contents in intact and PX pigeons subjected to functional manipulations of adrenal in the breeding phase
(* Significant at $p < 0.05$; values are $\bar{x} \pm$ SEM)

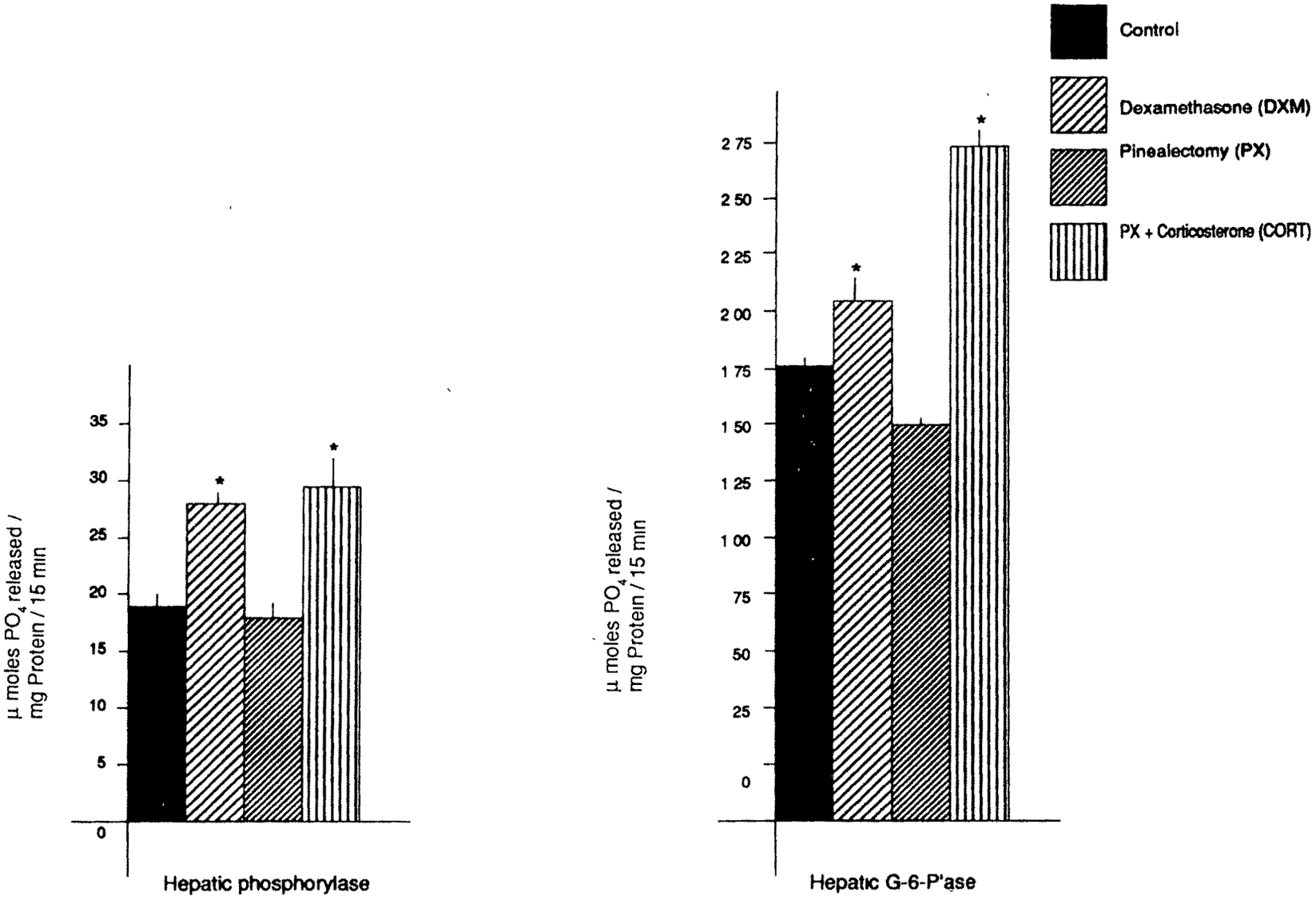

Fig.3B : Alterations in the activity levels of hepatic phosphorylase and G-6 p'ase of intact and PX pigeons subjected to functional manipulations of adrenals in the breeding phase.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

Treatments	Blood glucose (mg/dl)	Tissue glycogen (mg/100mg tissue)			Hepatic Phosphorylase (µg PO_4 released /mg Protein/15 min	Hepatic G-6-P'ase (µ moles PO_4 released/mg Protein/15 min)
		Liver	Muscle	Testis		
Control	192.67 ± 2.46	3.99 ± 0.14	1.24 ± 0.07	0.26 ± 0.02	19.56 ± 1.11	0.71 ± 0.01
CORT	211.72* ± 4.48	2.95 ± 0.35	1.38* ± 0.05	0.28 ± 0.02	24.20* ± 0.96	0.93* ± 0.14
PX	149.37 ± 1.25	2.74 ± 0.28	1.99 ± 0.11	0.25 ± 0.01	17.33 ± 1.30	0.30 ± 0.06
PX+CORT	129.22* ± 1.94	1.02 ± 0.01	1.83* ± 0.07	0.29 ± 0.02	23.98* ± 1.45	0.96 ± 0.03

Table IIIb. Alterations in blood glucose, tissue glycogen content and activity levels of hepatic phosphorylase and G-6-P'ase of intact and PX pigeons treated with corticosterone (CORT) in the regression phase.
(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

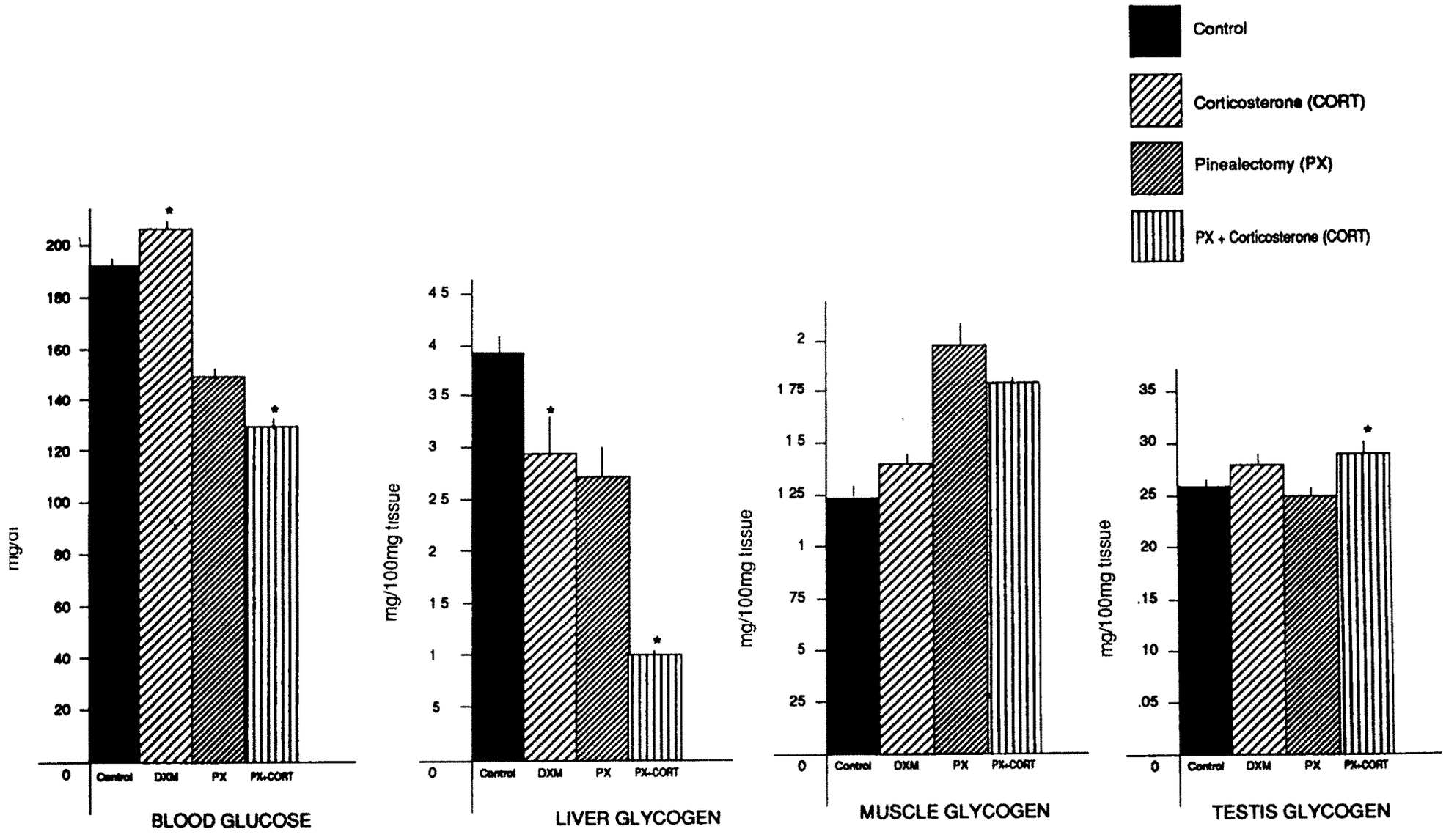

Fig. : 3 C Alterations in blood glucose and tissue glycogen contents of intact and PX Pigeons treated with corticosterone (CORT) in the regression phase
(* Significant at $p < 0.05$; values are $\bar{x} \pm$ SEM)

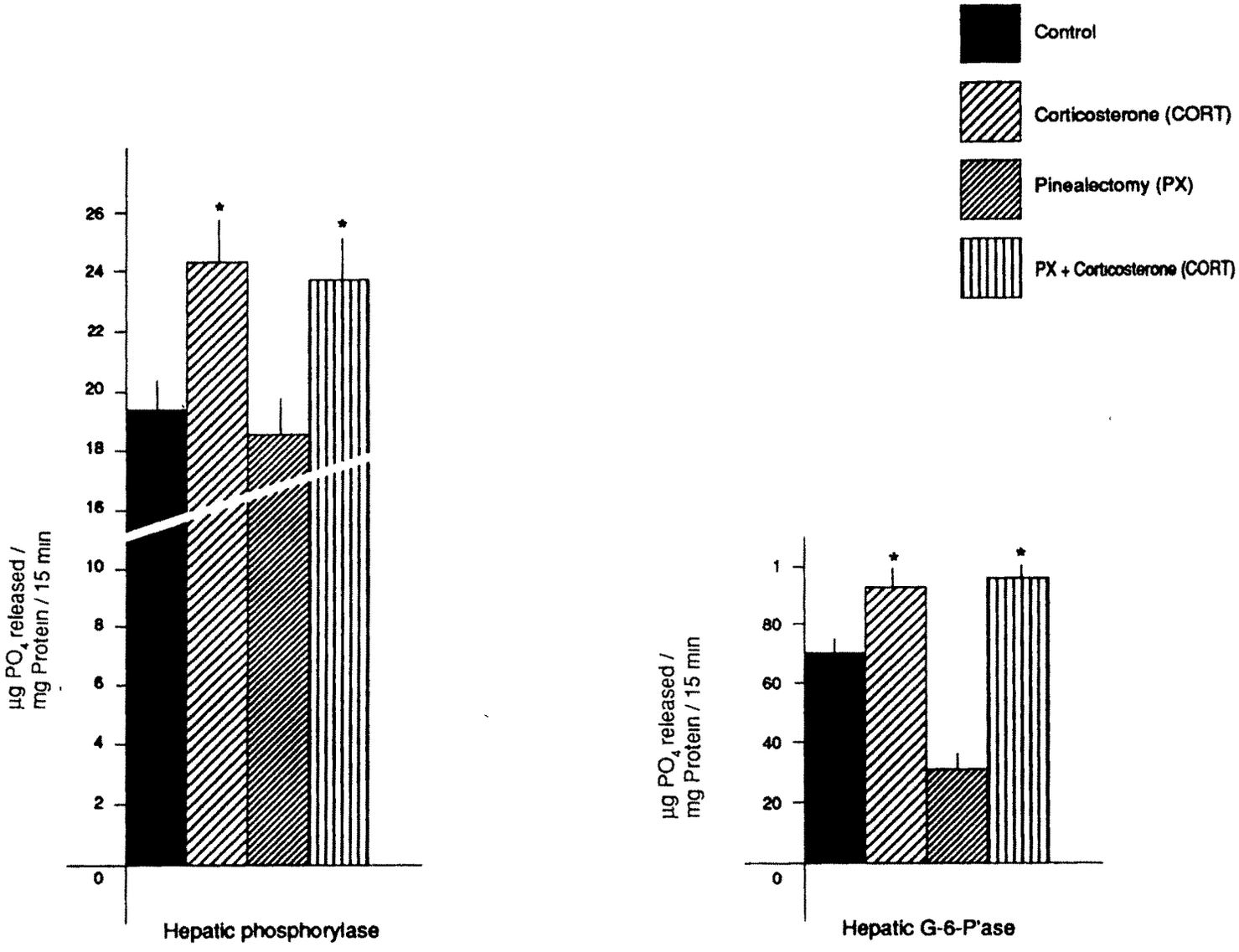

Fig 3D Alterations in activity levels of hepatic phosphorylase and G-6-P'ase of intact and PX pigeons treated with corticosterone (CORT) in the regression phase (* = Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

Treatments	Blood glucose (mg/dl)	Tissue glycogen (mg/100mg tissue)			Hepatic Phosphorylase (µg PO_4 released /mg Protein/15 min	Hepatic G-6-P'ase (µ moles PO_4 released/mg Protein/15 min)
		Liver	Muscle	Testis		
Control	158.29 ± 5.16	5.14 ± 0.30	1.84 ± 0.03	0.24 ± 0.01	13.82 ± 0.18	0.12 ± 0.004
CORT	172.18* ± 2.52	4.53* ± 0.14	2.62* ± 0.08	0.27 ± 0.02	17.78* ± 0.84	0.23* ± 0.03

Table IIIc. Alteration in blood glucose, tissue glycogen content and activity levels of hepatic phosphorylase and G-6-P'ase in the pigeons subjected to hypercorticalism in the quiescent phase.

(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

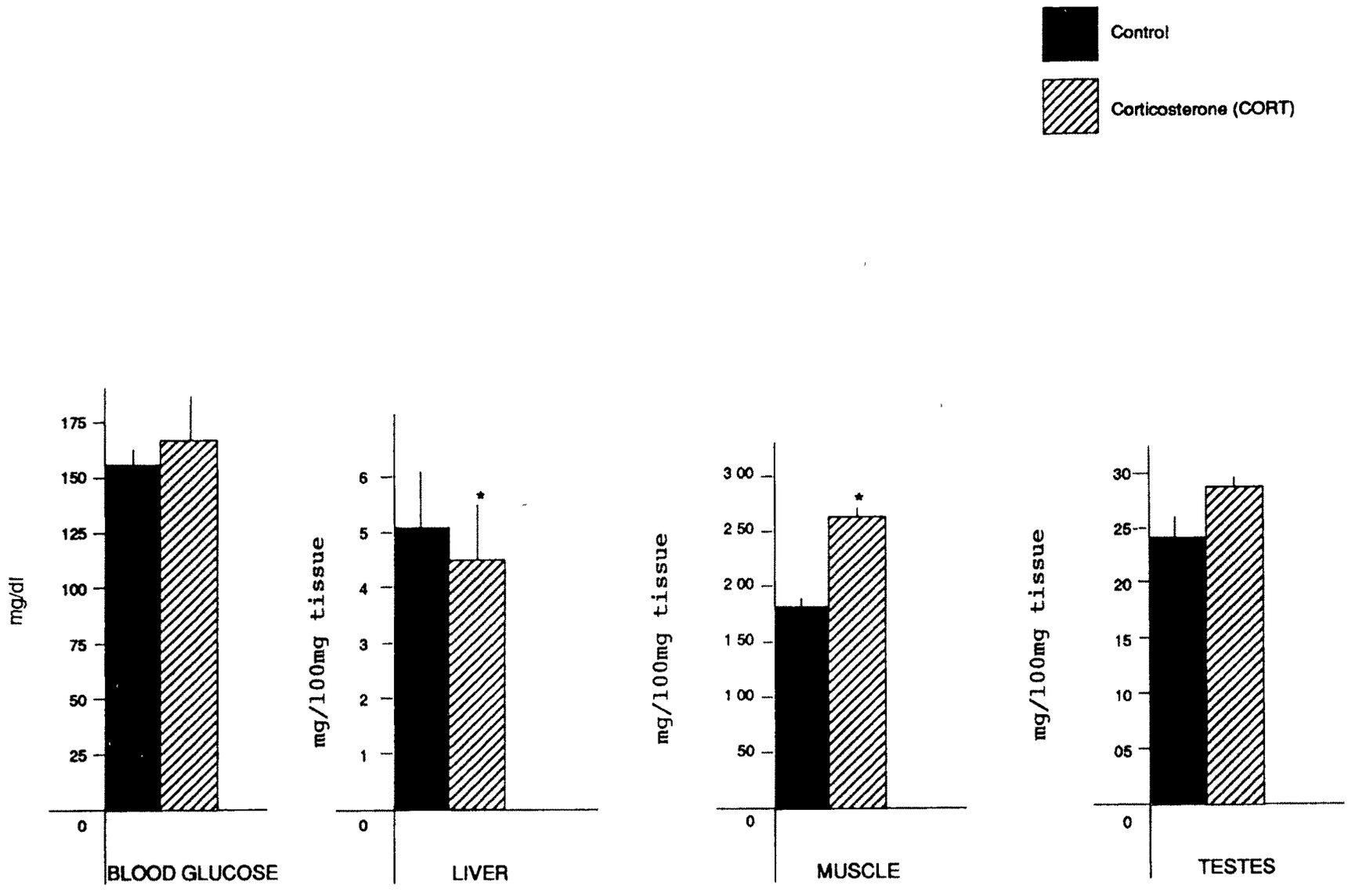

Fig. 3E Alterations in blood glucose and tissue glycogen contents of the pigeons subjected to hypercorticalism in the quiescent phase (* = Significant at $P < 0\ 05$; values are $\bar{x} \pm$ SEM)

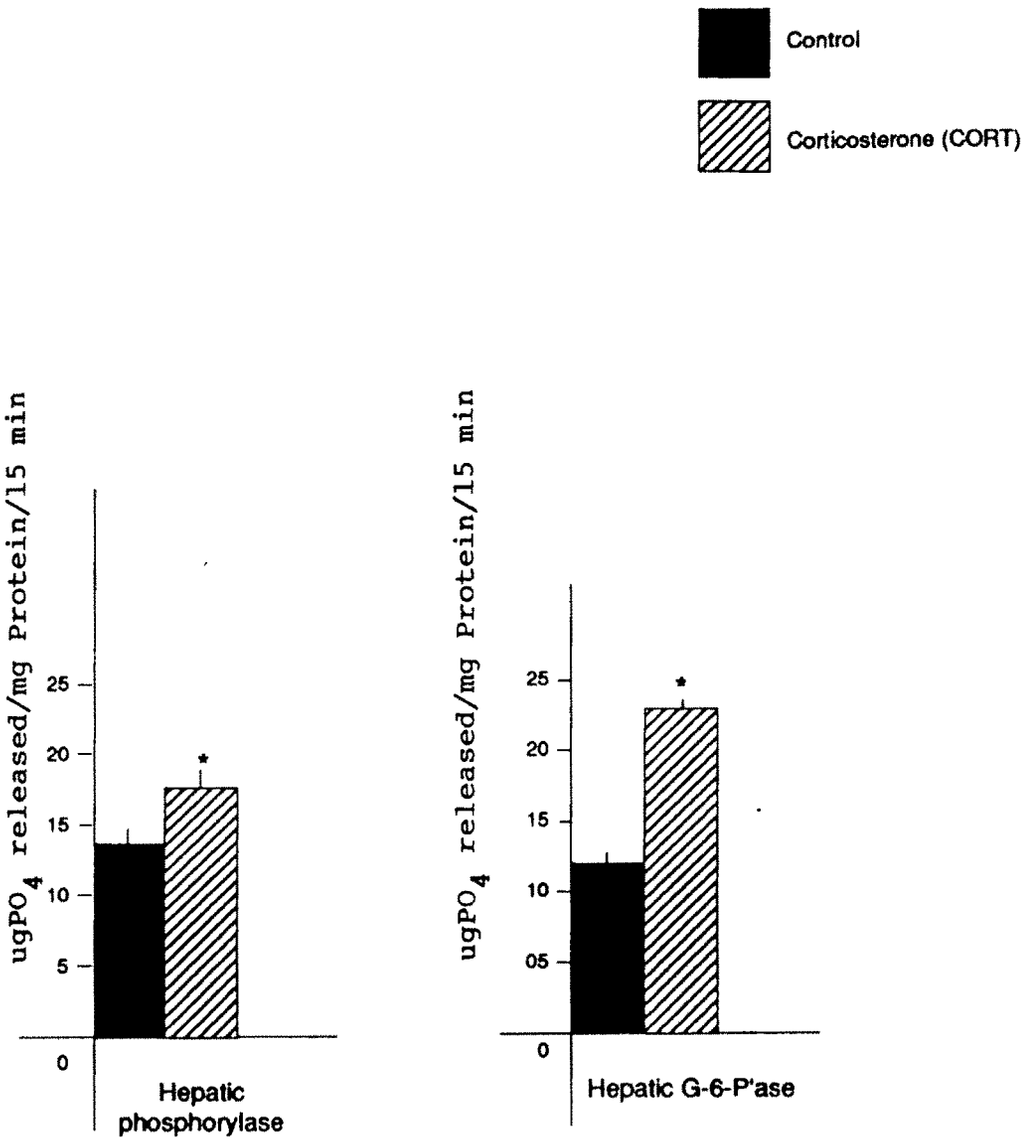

Fig 3F Alterations in the activity levels of hepatic phosphorylase and G-6-P'ase of pigeons subjected to hypercorticalism in the quiescent phase
(* Significant $P < 0\ 05$; values are $\bar{X} \pm$ SEM)

CORT treatment to intact birds during the regression and quiescent phases increased the muscle and testicular glycogen contents and decreased the hepatic glycogen content. (Tables III a to III c; Figs 3A, 3C, 3E)

Hepatic phosphorylase and G-6-P'ase activity.

Hepatic phosphorylase and G-6-P'ase activity were increased under all treatment schedules irrespective of the season. (Tables III a to III c; Figs 3B, 3D, 3F)

DISCUSSION.

The present studies on circannual variations in carbohydrate metabolism have revealed changes in metabolites and activity levels of enzymes, bearing some relationship with testicular cyclicity. Accordingly, the phase of testicular recrudescence in the immediate post-winter solstice period (January-February), was marked by significant hepatic glycogen depletion. Concomitantly, blood glucose level was increased and these changes were accompanied by increased hepatic phosphorylase and G-6-P'ase activity. The muscle and testis glycogen content was also at the lowest. Apparently, testicular recrudescence and establishment of full testicular functions require high energy input which is met by the carbohydrate reserves in the body including the gonads. The increasing tissue glycogen content and

decreasing blood glucose level, subsequent to the establishment of full testicular functions (March-April), suggest either decreased energy requirements or decreased utilization of carbohydrate reserves. Interestingly, the increase in tissue glycogen content which started from April, reached a maximal level in muscle and testis by August-September.; while the hepatic glycogen continued to show increasing content till a peak level was reached by December. The blood glucose level also remained in the lowest range between August & December. Obviously, the regression and non-breeding phases represent a phase of replenishment of carbohydrate reserves with reduced utilization of hepatic glycogen and plasma glucose. However, utilization of muscle glycogen as an energy source cannot be discounted as muscle glycogen content showed steady decline from September onwards till it attained the lowest level in February. Overall, the observed circannual variations indicate two significant points (1) the phases of testicular recrudescence and early breeding (January-April) are energitically demanding and is marked by depletion of body carbohydrate reserves and maintenance of high blood glucose level. (2) Muscle glycogen meets the low energy demand of the non-breeding and pre-recrudescent phases thereby implicating muscle as an important energy source in the annual budgetary provisions of this avian species.

Experiments involving functional manipulation of adrenocortical activity in intact and PX pigeons, which were primarily carried out keeping in mind the parallel pineal-adrenal-testes relationship, have revealed season specific effects of adrenal and pineal on carbohydrate metabolism. Accordingly, the increased liver, muscle and testis glycogen content and decreased blood glucose level obtained under induced adreno-cortical insufficiency are in confirmity with the earlier preliminary studies (Ayyar, 1987). These changes when compared with the observed circannual variations relate more closely with the changes occurring at the time of testicular regression (May-June), also marked by decreased serum CORT level (Chapter III). Moreover, increased blood glucose level and decreased hepatic glycogen content induced by CORT administration in the regression and non-breeding phases, are akin to changes occurring at the time of testicular recrudescence in the post-winter solstice period. Though the hepatic glycogen was found to be labile to the influence of CORT, the muscle and testis glycogen content appeared to be more resistant. The above observations indicate CORT to be one of the hormones involved in the circannual modulation of carbohydrate metabolism in relation to the testicular activity. In this respect, though CORT administration was able to alter the status of carbohydrate metabolism in the regression and non-breeding phase to that of recrudescent phase, it was

nevertheless without any effect in inducing testicular activation due to its inability to stimulate the inactive HHG axis as inferred earlier (chapter II and III).

In keeping with the previously established parallel pineal-testes axis (Ramachandran et al.,1987; Ramachandran and Patel, 1988) and parallel pineal-adrenal relationship (Patel et al., 1985, Ramachandran and Patel.,1986), PX induced alteration in carbohydrate metabolism assessed currently during the breeding and regression phases have shown changes which are neither in keeping with testicular activity nor with adrenal activity. In contradistinction to the increasing tissue glycogen content occurring either during the phase of testicular regression or during the experimentally induced hypocorticalism (Table IIIb; fig 3B), PX, induced tissue glycogen depletion despite bringing about testicular regression. These changes confirmed the earlier observations made in this laboratory in relation to PX (Ramachandran and Patel, 1987; Patel et al., 1988). The hypoglycemia and lowered tissue glycogen content induced by PX have been explained in terms of decreased hepatic glucose uptake, increased peripheral utilization and increased insulin release/sensitivity. (Patel and Ramachandran, 1992; Patel, 1993). A noteworthy observation of the present study is the potentiating influence of CORT on the PX induced alterations in carbohydrate metabolism. Apparently

alterations in intact or PX pigeons is differential with respect to carbohydrate metabolism. The inability of CORT to bring about the expected changes in carbohydrate metabolism in PX birds suggests the requirement of an optimum level of melatonin for CORT action. Moreover, CORT seems to potentiate PX effects involving hepatic glucose uptake, peripheral utilization as well as insulin sensitivity in the absence of melatonin. In the absence of any studies of this type a detailed constructive discussion of the present observations is impossible.

However, it could be inferred that there exists a subtle interrelationship between pineal secretions and CORT and that the normal actions of CORT occur in a background of an optimum level of melatonin. Overall, it can be concluded from the present study that -

(1) Circannual variations in carbohydrate metabolism occur in relation to testicular activity.

(2) Corticosterone has an important role in modulating carbohydrate metabolism.

(3) There exists an hitherto unknown relationship between CORT and melatonin.