CHAPTER 6

SEASONAL QUANTITATIVE CHANGES IN SODIUM, POTASSIUM AND CALCIUM IONS AND WATER CONTENT OF NORMAL AND PINEALECTOMIZED PIGEON, *COLUMBA LIVIA*.

In all physiological fluids and body cells, electrolytes such as sodium, potassium and calcium are the physiologically valuable cations. These cations play an important role in membrane polarization and permeability due to changes in their concentration inside and outside the cells. In the extracellular fluid, sodium is the major component of the monovalent cations, whereas potassium is the major one of the intracellular fluid. Sodium, potassium and calcium ions of the membrane influence the excitability of nerves and muscle cells. A large number of enzymic reactions are partly dependent upon these electrolytes (Colombo and Marcus, 1963; Ebashi *et al.*,1965; Diehl and Jones, 1966). Calcium is essential as a coupling factor between excitation and contraction in all muscle cells and between stimulus and secretion in certain endocrine secretions

as in ADH release by nerve impulses (Hasselback and Makinose, 1963; Hasselback, 1964; Weber, 1966; Weber et al., 1966).

In birds with seasonal reproductive cycle, requirement of different metabolites vary during different phases of gonadal activity. The changes in the concentration of sodium and potassium ions influence water balance in different cells and tissues. In the present investigation, at attempt has been made to study water content, concentration of monovalent cations -sodium and potassium - and the bivalent cation - calcium - in liver, muscle and adipose tissue and serum of the normal and pinealectomized feral blue rock pigeons, Columba livia, during the three reproductive phases.

MATERIALS AND METHODS

Normal, sham-operated and pinealectomized wild pigeons of various experimental groups as outlined earlier (chapter 1) were brought from the aviary and sacrificed. The tissues were taken, blotted free of blood and tissue fluids and homogenized in cold distilled water. Small pieces of liver, pectoral muscle and adipose tissue were taken for determining water content. Tissues were blotted properly to remove traces of tissue fluid, transferred to weighing bottles and dried. The difference in the weight after

complete drying was taken as the weight of water. The values are expressed as mg/100 ml fresh tissue. Dry lipid free pieces of liver, breast muscle and adipose tissue and blood serum were taken for estimation of inorganic cations (Na^+, K^+, and Ca^{++}) using 'EEL' flame photometer (methods described in Manual). Ionic concentrations in blood and tissues were expressed in terms of mg/100 ml of serum and mg/100 g dry lipid free tissue respectively.

RESULTS

Alterations in water content, sodium, potassium and calcium ion content in different tissues are presented in table 1 to 4 and figures 1 to 4.

SEASONAL CHANGES IN NORMAL BIRDS:

Water content in the liver and muscle was nearly same and very high than that of adipose tissue throughout the reproductive cycle. During the regression period water percentage in the liver and muscle was comparatively more. Both these tissues maintained reduced but the constant water content during recrudescence and breeding. Water content in adipose tissue was low in the regression period, from which it increased gradually through recrudescence and attained the maximal content during the breeding period.

Sodium content was highest in serum followed by adipose tissue, liver and muscle, in that order. Average Na^+ content for the three phases showed minimal concentration during breeding in serum,liver and muscle and maximal during

EXPLANATIONS FOR FIGURES

Figs. 1 to 4 : Histograms showing water content, sodium, potassium and calcium ion content in three different phases of reproductive activity, at three different intervals post-pinealectomy.

Fig. 1a - Water content in liver

Fig. 1b - Water content in muscle

Fig. 1c - Water content in adipose tissue

Fig. 2a - Sodium ion content in serum

Fig. 2b - Sodium ion content in liver

Fig. 2c - Sodium ion content in muscle

Fig. 2d - Sodium ion content in adipose tissue

Fig. 3a - Potassium content in serum

Fig. 3b - Potassium content in liver

Fig. 3c - Potassium content in muscle

Fig. 3d - Potassium content in adipose tissue

Fig. 4a - Calcium content in serum

Fig. 4b - Calcium content in liver

Fig. 4c - Calcium content in muscle

Abbreviations :

S - Sham operated.
N - Normal unoperated.
P - Pinealectomized.

FIG. 1a

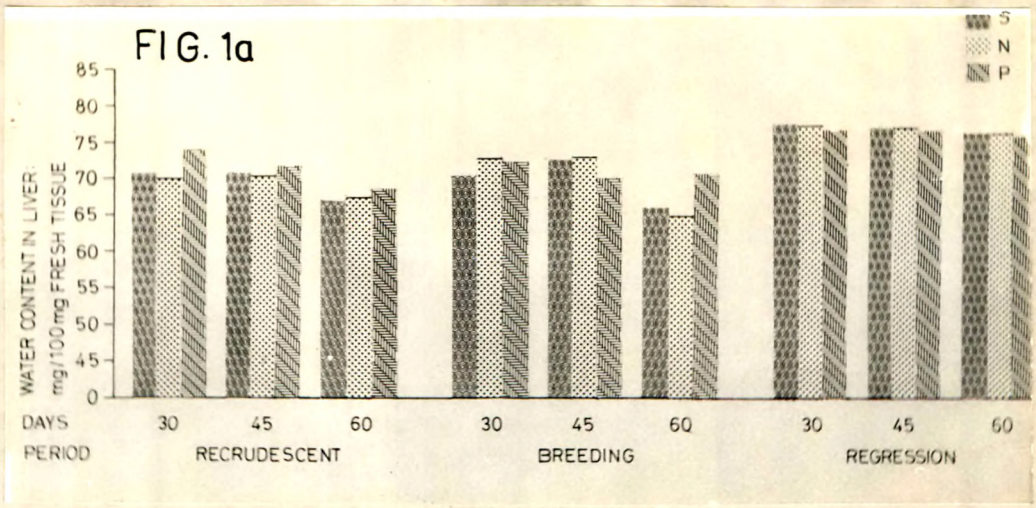


FIG. 1b

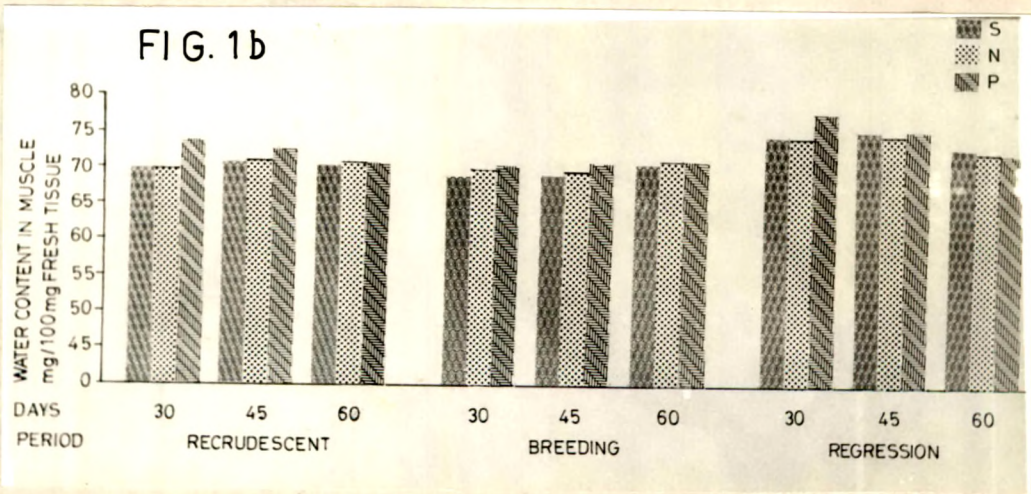


FIG. 1c

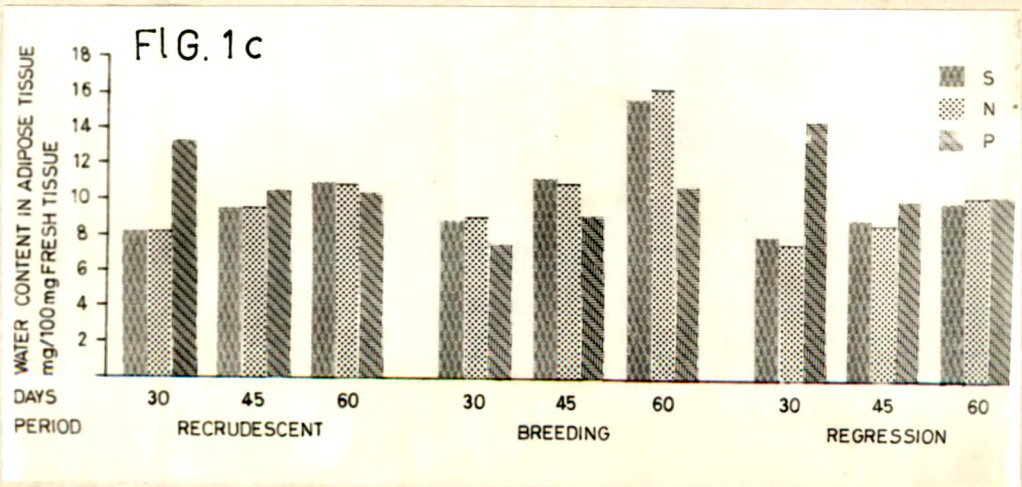


FIG 2a

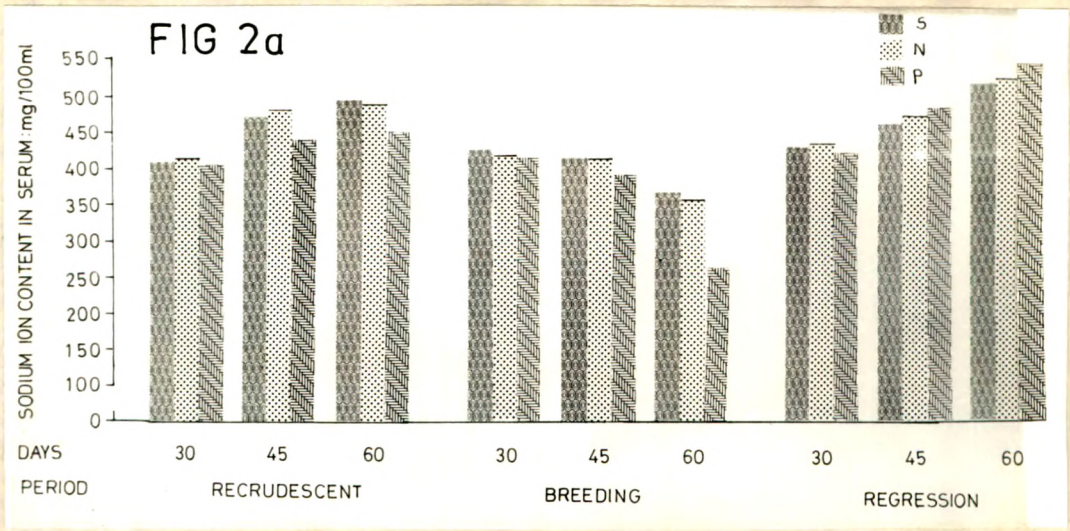

SODIUM ION CONTENT IN SERUM mg/100ml
550
500
450
400
350
300
250
200
150
100
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 2b

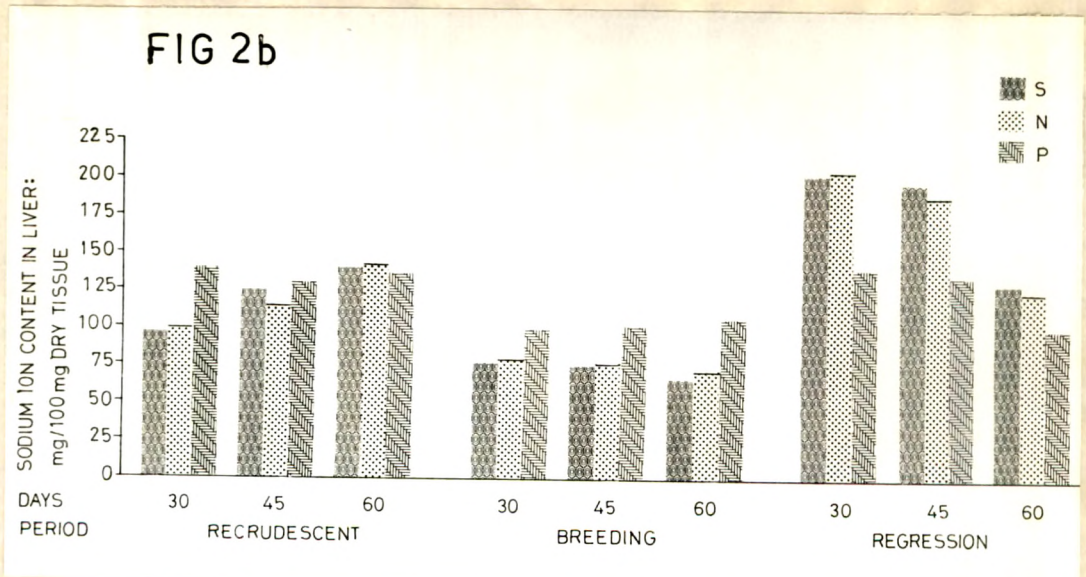

SODIUM ION CONTENT IN LIVER:
mg/100mg DRY TISSUE
225
200
175
150
125
100
75
50
25
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 2c

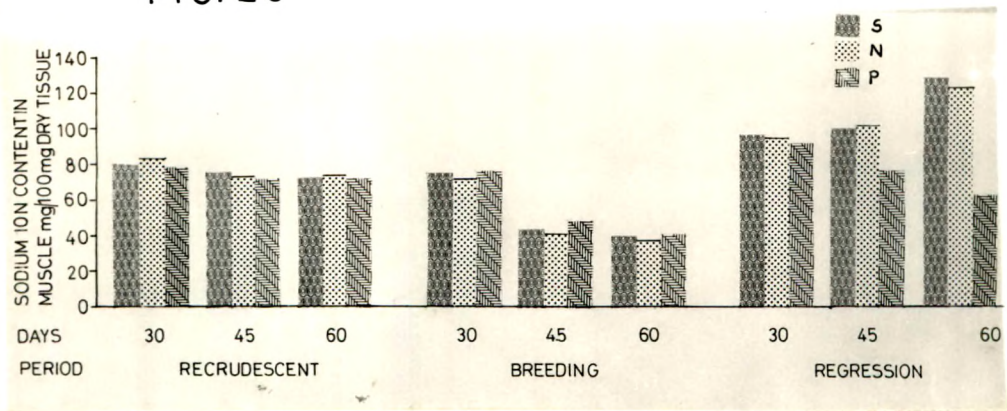

SODIUM ION CONTENT IN MUSCLE mg/100mg DRY TISSUE
140
120
100
80
60
40
20
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 2d

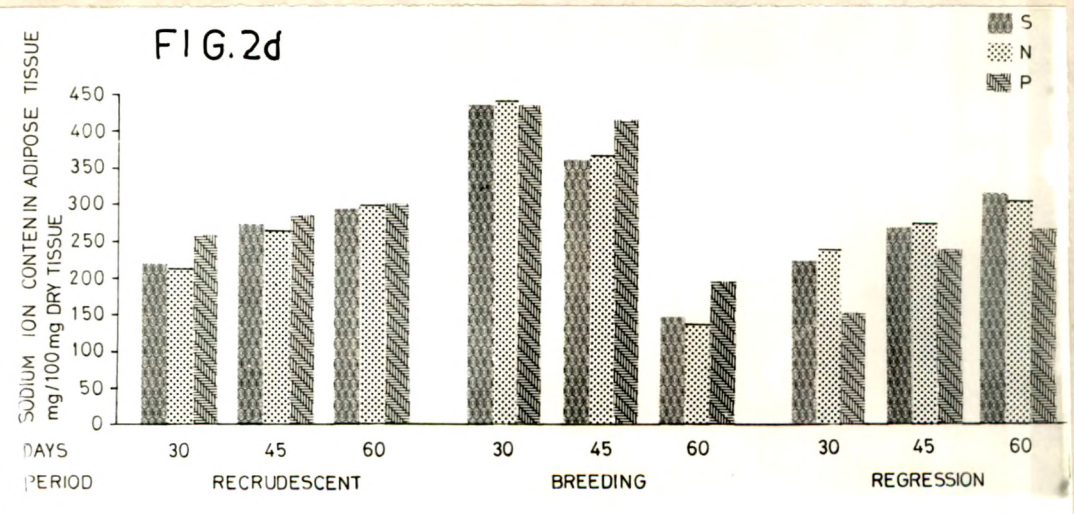

SODIUM ION CONTENT IN ADIPOSE TISSUE mg/100 mg DRY TISSUE
450
400
350
300
250
200
150
100
50
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3a

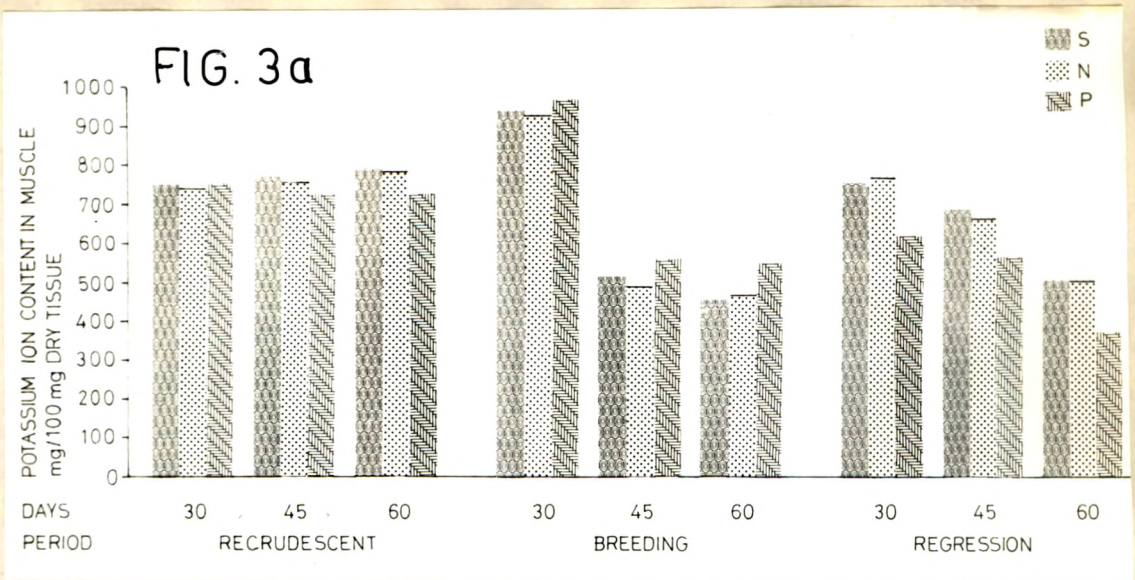

S
N
P
POTASSIUM ION CONTENT IN MUSCLE
mg/100 mg DRY TISSUE
1000
900
800
700
600
500
400
300
200
100
0
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3b

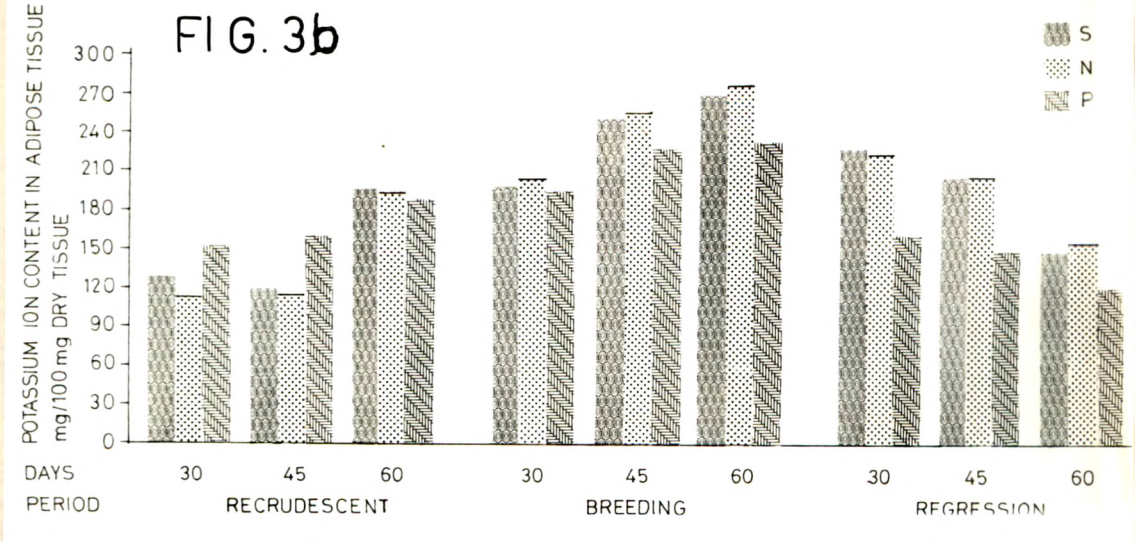

S
N
P
POTASSIUM ION CONTENT IN ADIPOSE TISSUE
mg/100 mg DRY TISSUE
300
270
240
210
180
150
120
90
60
30
0
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3c

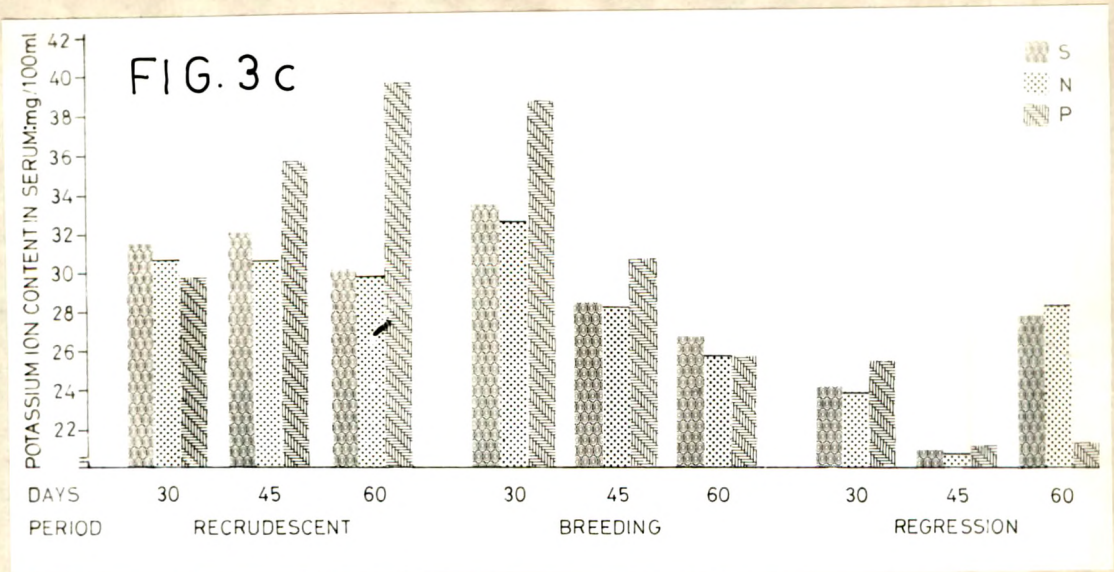

POTASSIUM ION CONTENT IN SERUM:mg/100ml
42
40
38
36
34
32
30
28
26
24
22
S
N
P
DAYS 30 45 60 30 45 60 30 45 60
PERIOD RECRUDESCENT BREEDING REGRESSION

FIG. 3d

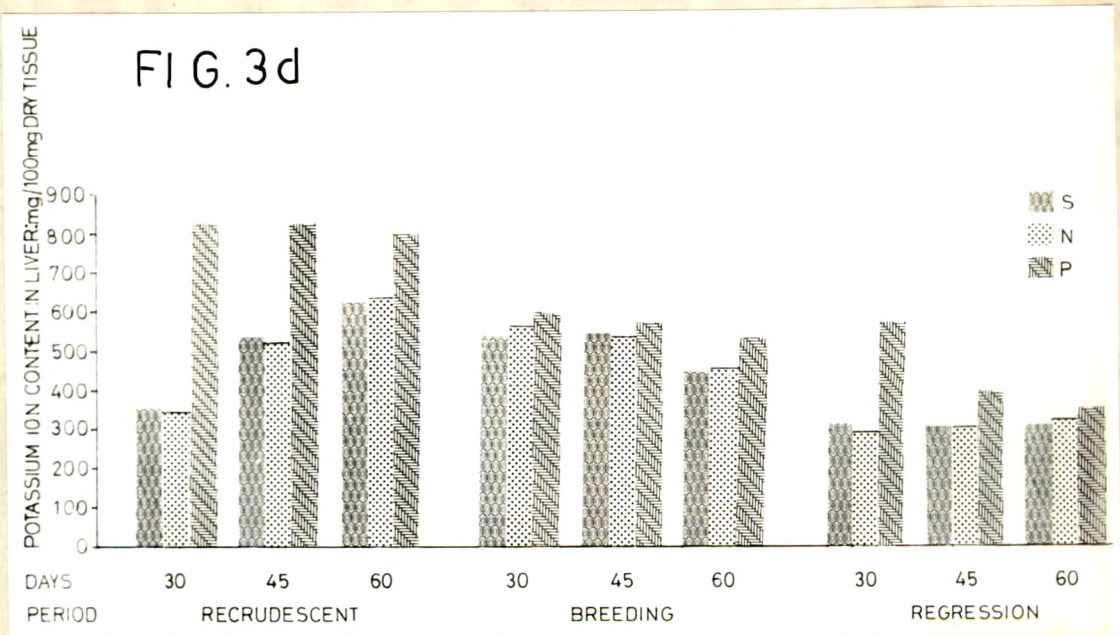

POTASSIUM ION CONTENT IN LIVER:mg/100mg DRY TISSUE
900
800
700
600
500
400
300
200
100
0
S
N
P
DAYS 30 45 60 30 45 60 30 45 60
PERIOD RECRUDESCENT BREEDING REGRESSION

FIG 4a

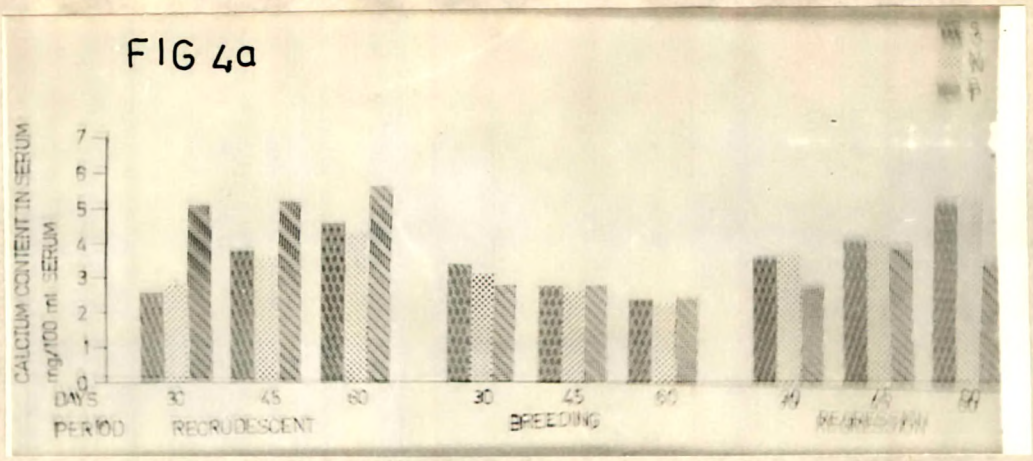


FIG 4b

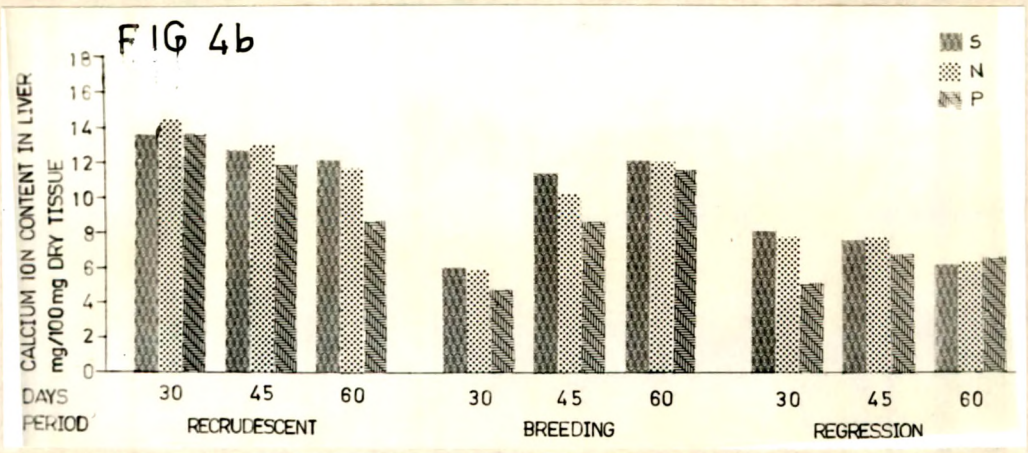
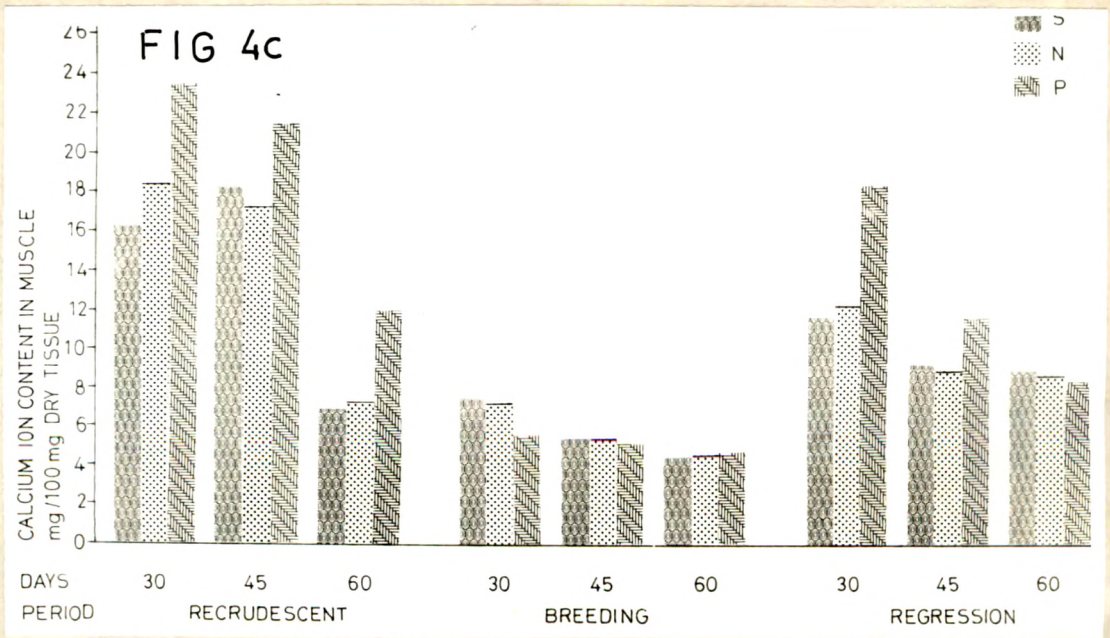


FIG 4c



the regression phase. Adipose tissue Na^{+} content was however maximal during breeding and lowest during recrudescence with a slightly higher content during regression. K^{+} ion content of serum and liver was higher during recrudescence and breeding with the maximal levels being during recrudescence in serum, and in liver during breeding. Minimal levels in both were recorded during regression. In the case of muscle, K^{+} ion content was minimal during breeding and maximal during recrudescence. However, K^{+} ion content of adipose tissue was maximal during breeding and minimal during recrudescence. Though the average K^{+} ion content of muscle was minimal during breeding period, taken as a whole, the highest K^{+} content was however recorded during the early breeding period. Average Ca^{++} content during each reproductive phase as a whole showed least amount of Ca^{++} in serum and muscle during the breeding period, and in liver, during regression. Whereas the maximal Ca^{++} content in serum was recorded during the regression phase, it was so in liver and muscle during the recrudescent phase.

CHANGES DUE TO PINEALECTOMY:

Water content showed some change only during the recrudescent phase in all the three tissues. An increase was recorded in all the cases. Breeding and regression did not produce any change in water content in liver and muscle. However, adipose tissue was affected by PX in all the three

phases. Recrudescence and regression showed increased water content, while in the breeding phase it was significantly decreased.

PX induced definite alterations in the Na^+ content of serum, liver, muscle and adipose tissue. Serum content of Na^+ was decreased during both recrudescence and breeding phases, while in the regression phase it was in the control range. Both liver and adipose tissue showed increased Na^+ content in the PX condition during recrudescence and breeding and, reduced content during regression. In the case of muscle, breeding and regression phases depicted similar changes. However, during recrudescence, the Na^+ content was again decreased mainly in the PX 30 and PX 45 animals.

Pinealectomy had no effect on the K^+ content in serum during the regression period. But during the recrudescence and breeding, K^+ content of serum was increased. In the liver, the content of K^+ was significantly elevated during all the three phases with a maximal one during recrudescence and a minimal one during breeding. K^+ content of muscle was reduced during regression and recrudescence in that order and increased during breeding. Adipose tissue presented a different picture with increase during recrudescence, and reduction during regression and breeding in that order.

Ca^{++} ion content in serum increased significantly in all PX birds of recrudescent phase. During the breeding phase, PX state tended to show a decreased serum Ca^{++} content. This reduction became more significant during regression. The liver Ca^{++} content was lowered in all the three phases of reproductive cycle. PX condition increased the muscle Ca^{++} content highly during the recrudescent and regression periods in that order. During the breeding phase, Ca^{++} content in muscle was lowered slightly.

DISCUSSION

In general, the changes in the Na^{+} and K^{+} content in blood and tissues appear to be inversely reciprocal. In order to maintain the osmotic balance, Na^{+} and water are known to move in or out of tissues in conjunction with each other. This is rather obvious form the parallel changes recorded herein for both Na^{+} and water contents in the tissues studied. Recrudescence and breeding are marked by decreasing Na^{+} ion content and increasing K^{+} ion content in blood, liver and muscle of wild pigeons. K^{+} entry into tissues is known to facilitate entry of glucose and other metabolites (Pilo and Patel,1978). In this context, the presently noted K^{+} ion content of liver and muscle during recrudescence and breeding might by bringing about such facilitative movement of metabolites set up the 'climate' for reproduction. Increased K^{+} content also denotes higher

metabolic activity. Reduced metabolic activity during regression is denoted by the reversed changes in Na^+ and K^+ contents which in turn is marked by elevated Na^+ / K^+ ratio. Altered physiological state of the pigeons during recrudescence and breeding is indicated by the lowered Na^+ / K^+ ratio in serum as well as in liver and muscle. Adipose tissue has shown a different pattern from that of liver and muscle. Na^+ and K^+ ions of adipose tissue were nearly equal during regression with a slightly lower K^+ content. During recrudescence, there was a slight fall in Na^+ ion content and pronounced depletion of K^+, thereby increasing Na^+ / K^+ ratio. This coupled with the previous observation of unaffected lipid content (chapter 5) might indicate the protective influence exerted by the ionic balance in preventing lipolysis during recrudescence. However, during breeding, both the ions were drastically increased with a fall in Na^+ / K^+ ratio leading probably towards a permissive action on lipolysis as observed in chapter 5.

The control of Ca^{++} ion in the blood is mainly brought about by the influence of a number of factors such as parathormone, calcitonin, blood pH, serum proteins, vitamin D, estradiol, estrogen etc. Changes in the activity of one organ produces various influences in other parts of the body, despite this, the Ca^{++} content of blood remains

remarkably constant (Simkiss, 1967). Very high content of hepatic calcium in the recrudescent period noted in the present study might represent increased mobilization and storage to meet the ensuing reproductive requirements. Fall in hepatic Ca^{++} content during breeding alongwith continuous decrease in blood Ca^{++} level throughout recrudescence and breeding, indicates utilization of Ca^{++} in events associated with reproduction. The major function of Ca^{++} in muscle is to serve as an intracellular coupling factor for contraction. During winter (recrudescent period),muscle seems to function more actively to generate heat to withstand low temperature. Perhaps, the high Ca^{++} level observed at this period might account for the same. The breeding period of wild pigeons corresponds to increasing environmental temperature and probably as a result Ca^{++} concentration decreased. Significantly, during regression, once again Ca^{++} level in muscle increased probably in relation to decreasing environmental temperature. It is quite likely that the adaptive alterations in Na^{+} , K^{+} and Ca^{++} content of blood and the tissues recorded herein during annual reproductive cyclicity of wild pigeons are mediated by the adrenal and parathyroid hormones.

Inability to increase cortisol secretion could occur due to damaged adremal cortex or suppression of secretion of ACTH. This results in rise of serum K^{+} and fall in

serum Na^+ (Keele and Neil,1971). Similar effect on serum Na^+ and K^+ concentrations was observed in PX birds. This suggests that PX condition decreases either ACTH or cortisol directly or indirectly. It is known that cortisol deficiency leads to hypoglycemia, higher sensitivity to insulin and lowering of hepatic glycogen (Holmes and Phillips, 1976). Low liver glycogen (chapter 3) and low blood glucose (chapter 2) noted in PX birds during recrudescence and breeding are supplimentary to the effect of cortisol deficiency on Na^+ and K^+ content in serum. Pinealectomy in rats has provided contradictory effects in serum Na^+ content, with a decrease being observed by Karppanen et al. (1970) and an increase reported by Tanner and Hungerford (1962) and Karppanen and Vapaatalo (1971). In contrast, PX birds showed elevated hepatic and adipose tissue Na^+ content during recrudescence and breeding, and muscle Na^+ content during breeding. Thus, increased tissue Na^+ content may have some inhibitory influence on the adaptive metabolic alterations reported to occur in conjuction with gonadal recrudescence and breeding activities. Rather unexpectedly, hepatic K^+ content also showed an increase which may bear correlation with the low hepatic glycogen content (chapter 3). The increased tissue Na^+ content and previously reported loss of phospholipids in PX conditions (chapter 5) may also have some relation. Decreased muscle K^+ content during all the three phases together with decreased Na^+ content during

recrudescence and regression signifies probable sub-optimal muscle activity and metabolism. The generally reversed trend of changes observable during regression is once again an indication of the season specific interactions of pineal with other endocrines. Loss of Na^+ coupled with hyperkelemia in blood are effects compatible with aldosterone insufficiency. Though it is generally accepted that loss of serum Na^+ is paralleled by increased urinary excretion, it is not clear whether increased tissue retention of Na^+ and K^+ observed presently could also be attributed to hypo-aldosteroidism. Obviously, certain degree of adrenocortical insufficiency in association with pinealectomy in wild pigeon can be deduced. This is supported by the hyperkelemic effect in blood and liver as well as the observed alteration in weight of the adrenals (chapter 1). However, the K^+ content of muscle is lowered in all phases, and this together with the increased Ca^{++} content might be speak of some muscle dysfunctioning which is correlatable with the morphologically visible reduced muscular activity in PX pigeons. Ca^{++} influx from extracellular fluids is known to be promoted by K^+ (Cunnane et al., 1980). On this basis, an inverse relationship between the two cations can be inferred. Such a relationship is the feature in the various tissues of PX pigeons. It is rather enigmatic, how such ionic alterations could be brought about by pinealectomy. Number of unknown factors could be purported to bring about tissue ionic changes, and

pinealectomy might affect any of the factor/s. Moreover, with the reported involvement of pineal, and Na^+ and K^+ in thermoregulation (Ralph et al., 1979; Den Boer and Edens, 1981), a common axis of pineal - cations - body temperature could be deduced from the present observations.