CHAPTER III

SEASONAL VARIATIONS IN CERTAIN BIOCHEMICAL PARAMETERS OF KIDNEY AND INTESTINE AS RELATED TO REPRODUCTIVE CYCLES

Involvement of Ascorbic acid (AA) in reproduction is now a well documented fact, as has already been considered in Chapter II. One of the noteworthy reports concerned pertains to the involvement of dehydroascorbic acid (DHA) in the activation of Δ^5-3β-hydroxysterod dehydrogenase (3β-HSDH), one of the key enzymes of steroid metabolism (Biswas, 1969; Biswas and Deb, 1970). A close relationship between gonadal functions and AA has been amply reviewed by Sebrell and Harris (1967). It is therefore apparent that AA is an important vitamin as far as the reproductive functions are concerned.

As explained in Chapter II, a phylogenic trend in the sites of AA synthesis has been reported among birds indicating that kidney and/or liver are the sites for the AA synthesis in different groups of birds (Roy and Guha, 1958; Ray Chaudhuri and Chatterjee, 1969).

Though dietary AA is not required in chicken as all the enzymes responsible for AA synthesis are present in kidney, it is known to be beneficial in diet during certain conditions of "stress" when chicken are unable to synthesize adequate amounts. Addition of the vitamin is reported to mitigate the adverse effect of high environmental temperature (Pardue *et al.*, 1983), facilitating improvement of semen production in cocks and egg production in hens maintained in hot climatic conditions (Coates,1971).

Absorption of AA in chicken intestine was reported to be active when its concentration was low in ingested food and passive when it was high in diet (Nagorna-Stasiak *et al.*, 1986). Reduction in body fat in scurvy has been reported as early as 1929 by Nagayama and Tagaya. Later on several workers have reported on the relation between AA and cholesterol.

Cholesterol is known to be synthesized in the gastro-intestinal tract (G.I. tract) of rats and monkeys (Dietschy and Weis, 1971) and chicken (Yeh and Levellie, 1973). Cholesterol is also known to be absorbed as well as excreted by the intestine. It is excreted by G.I.tract as catabolic steroid products *viz.*, bile acids or as cholesterol itself (Smith *et al.*, 1985). Further, these authors have explained that the greater part of cholesterol from body is eliminated by two pathways, that is through conversion to bile acids via bile and through excretion of neutral sterols in the faeces. Catabolic products of steroid hormones are also eliminated via urine as conjugated derivatives. However, this pathway is not important as far as birds are concerned since the nature of urinary excretion is different. It is well established fact that cholesterol is converted to progesterone which in turn is converted to different androgens as well as oestrogens depending on the sex of the individual.

Bile, which is secreted by liver, and stored and concentrated in the gall bladder enters the intestine continuously, however, the rate of its release depends upon the presence of partially digested food in the intestine. Bile contains, bile salts, bile pigments and cholesterol, synthesized in liver. Bile acids, the C_{24} steroids with side chain of 5 carbon at C_{17}, are reabsorbed 2 to 3 times via enterohepatic circulation. Although reabsorption of bile acids is very efficient, a small fraction is always lost with undigested food, and to maintain this pool, some amount is replaced through hepatic synthesis. Unesterified cholesterol is a major biliary constituent. Much of the cholesterol is reabsorbed in the jejunum and returned to liver for recirculation by jejunal mucosa or excreted out. Glucosiduronates of other cyclic alcohols are also excreted in the bile and represent a major component coming from general metabolism (Martin, 1980).

The middle section of the intestine is known to be the most active site of fat absorption in the chicken (Renner, 1965), whereas Whitehead (1973) indicated that in the adults, fat can be absorbed in more proximal section of the small intestine.

Gurr and James (1971) described that before absorption, hydrolysis of esterified cholesterol to free cholesterol takes place in the intestine and subsequent reesterification occurs within the mucosal cells before being transported to liver. Further, they have also pointed out that reesterified cholesterol is incorporated into lipoprotein of chylomicrons passing on to the lymph. These authors have also discussed the possible role of liver in the resynthesis of ester cholesterol with changes in fatty acid composition and their release in plasma as components of lipoproteins.

Thus, kidney as well as intestine are involved in the metabolic handling of cholesterol and its metabolites. This probably happens to be indirectly influenced by reproductive phases.

Most of the above cited reports are based on mammalian studies or on domesticated avian species like chicken. Thus, it was thought desirable to investigate the changes taking place in AA, total lipid and cholesterol (total and esterified) in the kidney and intestine of two as yet less studied but closely related species of birds Bank myna *Acridotheres ginginianus* and Brahminy myna *Sturnus pagodarum* with respect to their reproductive cycles.

MATERIAL AND METHODS

As reported in earlier chapters, birds were acquired from a local animal dealer and sacrificed as early as possible to avoid effect of caging. Left kidney and proximal part of the intestine (jejunum) were taken out. Kidney was blotted free of tissue fluids, divided longitudinally in almost two equal parts of which one was utilized for estimation of AA and the other for total lipids, total cholesterol and esterified cholesterol. The part of intestine was split open so as to expose the lumen, repeatedly washed in cold saline and then utilized for the assessment of above parameters.

As described in Chapter II, AA was estimated by employing the method of Roe (1954) and total cholesterol and esterified cholesterol after Crowford (1957). Total lipids were extracted in alcohol : ether (3 : 1) mixture and measured gravimetrically as described in Chapter I.

RESULTS

The variations in the levels of AA, total lipids, total cholesterol and esterified cholesterol are presented in the Table 3.1 and Fig. 3a for male birds and Table 3.2 and Fig. 3b for female birds.

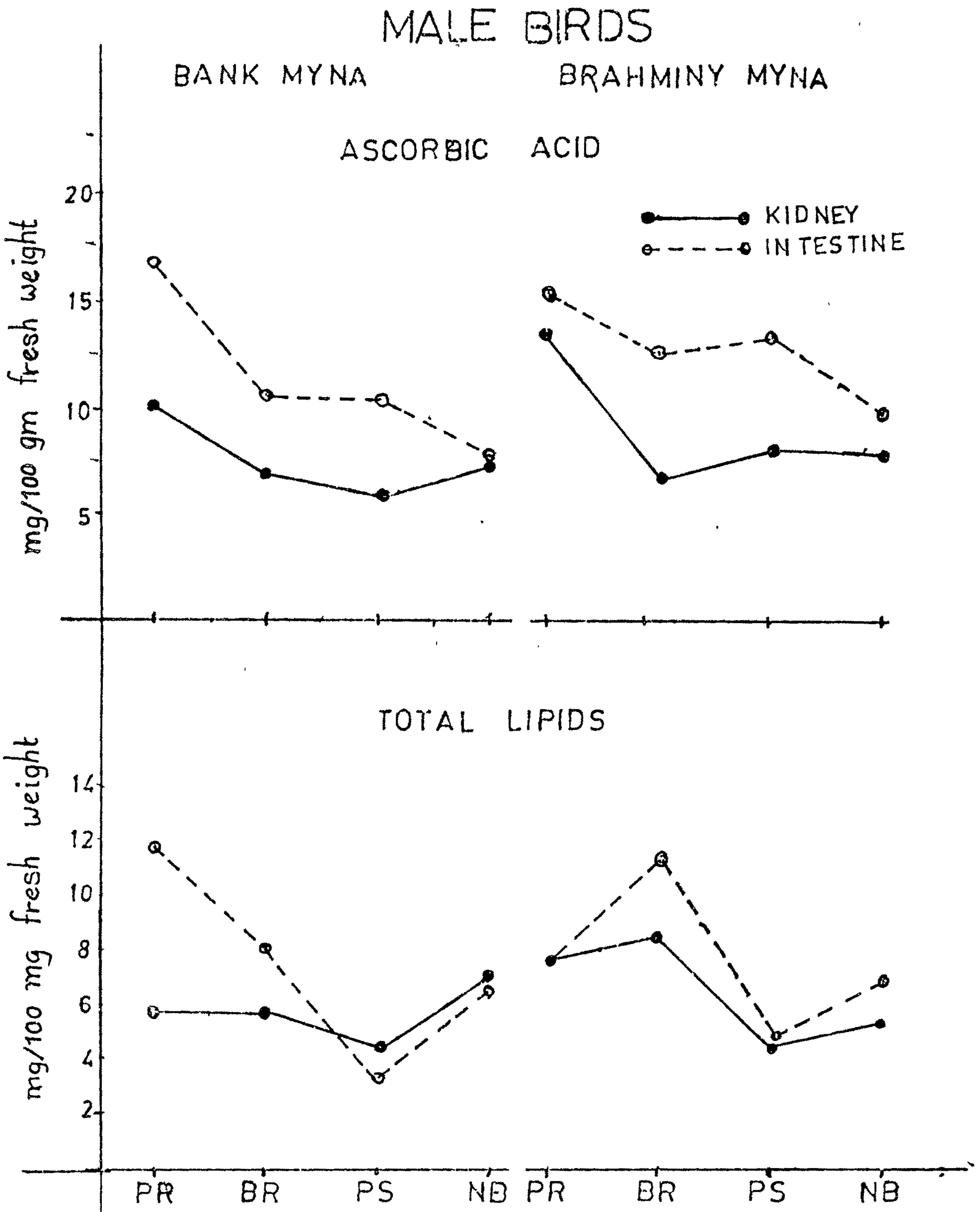
Table 3.1

Seasonal variations in some parameters of kidney and intestine in two species of myna as related to male reproductive cycle.

Parameters	Ascorbic Acid@		Total Lipid@@		Cholesterol@@			
					Total		Esterified	
	Kidney	Intestine	Kidney	Intestine	Kidney	Intestine	Kidney	Intestine
Seasons				Bank myna				
PR	10.168 ±1.46	16.422 ±1.68	5.609 ±0.553	11.798 ±3.19	0.459 ±0.03	0.31 ±0.03	0.232 ±0.02	0.15 ±0.01
BR	6.904 ±1.23	10.658 ±1.76	5.791 ±0.905	8.04 ±1.59	0.503 ±0.06	0.396 ±0.07	0.269 ±0.03	0.197 ±0.01
PS	5.88 ±0.76	10.338 ±1.09	4.708 ±0.609	3.658 ±0.498	0.308 ±0.03	0.282 ±0.04	0.214 ±0.05	0.161 ±0.02
NB	7.149 ±0.43	7.798 ±0.43	7.031 ±0.261	6.753 ±0.53	0.347 ±0.02	0.414 ±0.04	0.292 ±0.03	0.195 ±0.01
				Brahminy myna				
PR	13.277 ±1.197	15.271 ±1.585	7.621 ±0.571	17.603 ±0.759	0.605 ±0.01	0.42 ±0.02	0.209 ±0.02	0.165 ±0.01
BR	6.701 ±0.54	12.687 ±0.66	8.577 ±0.762	13.303 ±1.082	0.459 ±0.02	0.391 ±0.01	0.274 ±0.01	0.206 ±0.01
PS	7.758 ±1.17	13.44 ±1.89	4.244 ±0.368	4.999 ±0.713	0.358 ±0.01	0.289 ±0.04	0.16 ±0.03	0.141 ±0.02
NB	7.628 ±0.55	9.698 ±0.81	5.717 ±1.007	6.923 ±0.774	0.904 ±0.09	0.525 ±0.71	0.451 ±0.05	0.248 ±0.03

Values expressed as Mean±S.E.

@ - mg/100 gm fresh tissue, @@ - mg/100 mg fresh tissue weight



MALE BIRDS
BANK MYNA
BRAHMINY MYNA
ASCORBIC ACID
KIDNEY
INTESTINE
mg/100 gm fresh weight
20
15
10
5
TOTAL LIPIDS
mg/100 mg fresh weight
14
12
10
8
6
4
2
PR
BR
PS
NB
PR
BR
PS
NB

Fig. 3a(i)

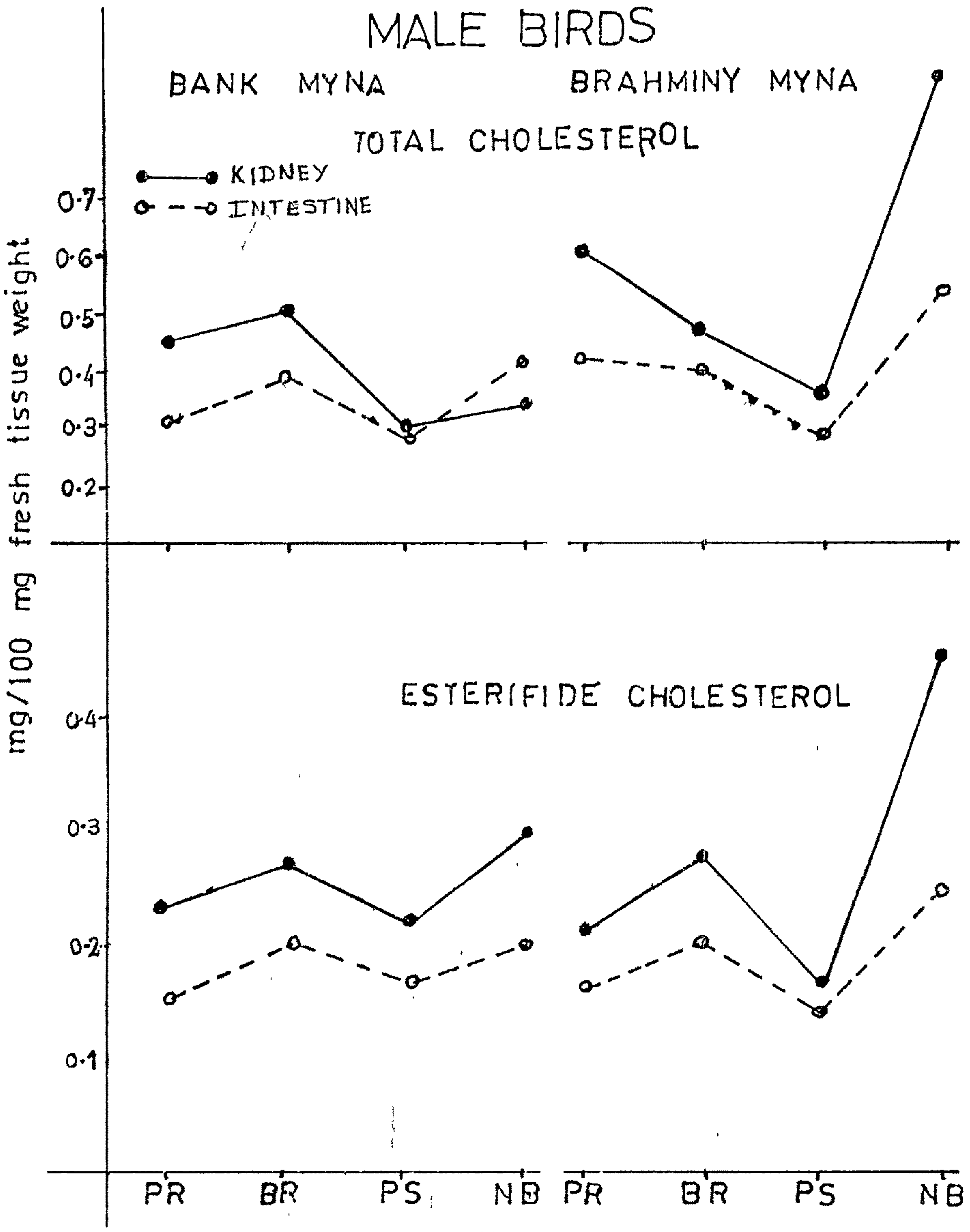

MALE BIRDS
BANK MYNA
BRAHMINY MYNA
TOTAL CHOLESTEROL
KIDNEY
INTESTINE
mg/100 mg fresh tissue weight
0·7
0·6
0·5
0·4
0·3
0·2
ESTERIFIDE CHOLESTEROL
0·4
0·3
0·2
0·1
PR
BR
PS
NB
PR
BR
PS
NB

Fig. 3a(ii)

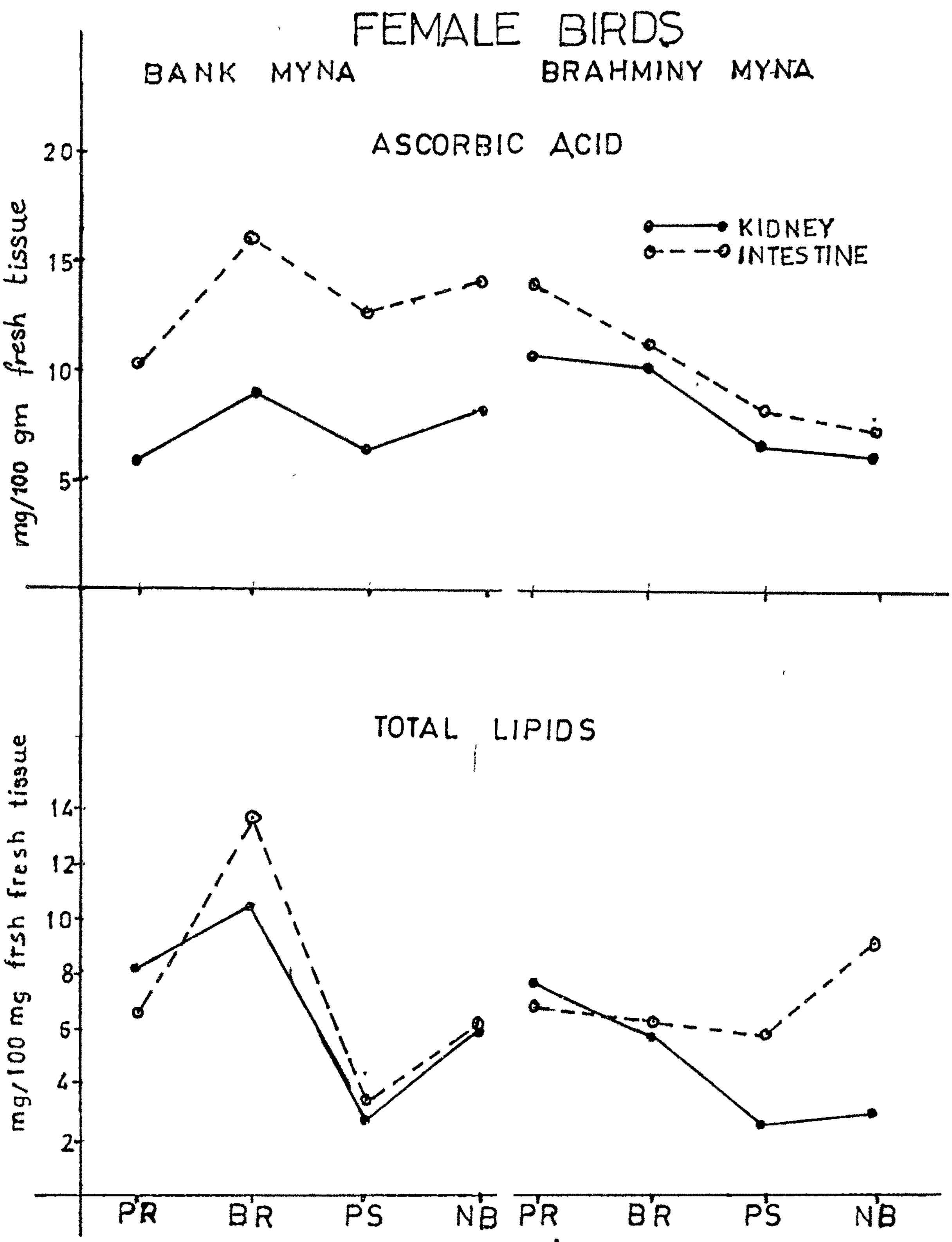

FEMALE BIRDS
BANK MYNA
BRAHMINY MYNA
ASCORBIC ACID
KIDNEY
INTESTINE
mg/100 gm fresh tissue
20
15
10
5
TOTAL LIPIDS
mg/100 mg frsh fresh tissue
14
12
10
8
6
4
2
PR
BR
PS
NB
PR
BR
PS
NB

Fig. 3b'(i)

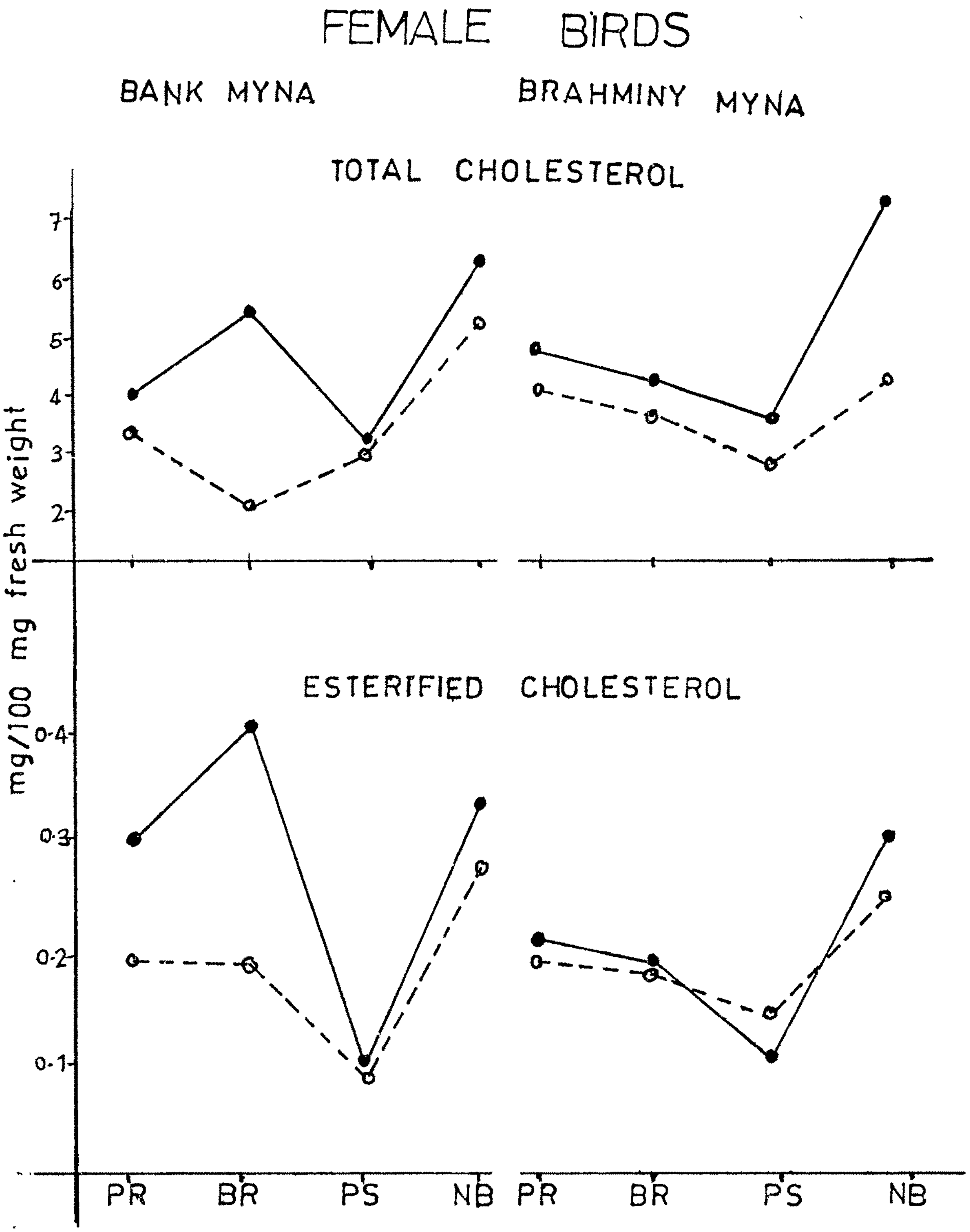

FEMALE BIRDS
BANK MYNA
BRAHMINY MYNA
TOTAL CHOLESTEROL
ESTERIFIED CHOLESTEROL
mg/100 mg fresh weight
7
6
5
4
3
2
0·4
0·3
0·2
0·1
PR
BR
PS
NB
PR
BR
PS
NB

Fig. 3b(ii)

Male birds : In male birds kidney and intestine showed almost similar patterns of the variations in both species, except for minor variation from PS to NB. AA level was found to be higher in intestine than in kidney in both the species. Highest AA levels were obtained during PR and lower AA levels during NB in both species.

In Bank myna intestine had higher total lipids during PR which decreased steadily from BR to PS and increased again during NB whereas in kidney lipid levels were lower as such and were maintained during PR and BR, with a marginal decrease during PS and an increase during NB. In the other species, total lipids were low during PR in both tissues, which increased during BR, again decreased during PS and reincreased during NB. In Bank myna TC was more or less maintained in both tissues from PR to BR whereas a decrease was obtained during PS and during NB it almost leveled off in case of kidney and increased in the intestine. In the other species, a steady decrease was obtained in total cholesterol level of both tissues from PR to BR to PS whereas a sharp increase was noted during NB in both the tissues that of the kidney attaining highest level of the cycle.

In Bank myna EC levels in both the tissues exhibited almost parallel patterns of variations and the NB levels were higher than PR levels. In the case of kidney and intestine the lowest values of annual cycle were recorded during PS. Regarding the variations in Brahminy myna in kidney and intestine EC contents, it can also be said to show parallel patterns of variations except for a far remarkable rise in the kidney than that of the intestine. Here also the NB levels were higher than those during PR.

Species-specific differences were obvious as far as the total lipid and TC levels were concerned throughout the annual cycle :-

1. In Bank myna both the tissues exhibited a gradual decrease in total lipid levels from PR to BR to PS. However, the decrease in intestinal total lipids was comparatively steeper.
2. In case of Brahminy myna, on the other hand, the total cholesterol levels exhibited a gradual decrease from PR to BR to PS.
3. Though the trends of variations in esterified cholesterol contents were similar in both species qualitatively, on quantitative basis the rise in the kidney of Brahminy myna was highly significant from PS to NB.

Female birds : The pattern of AA variation in both the tissues of Bank myna exhibited a parallel pattern with an increase from PR to BR and decrease from BR to PS and a slight increase from PS to NB. As opposed to this those of the Brahminy myna showed continually decreasing trend throughout. The species-specific differences were very obvious.

Total lipid levels in the intestine as well as kidney of Bank myna exhibited a distinct rise from PR to BR; that of the intestine being highly significant. In contrast to this both these tissues showed a slight loss of total lipid content in case of Brahminy myna. Variations obtained in total lipid content of kidney as well as intestine within a species can be said to show parallel patterns. Nevertheless species-specific differences were very obvious, the intestine and kidney of Bank myna recorded a significant rise from PR to BR whereas those of Brahminy myna exhibited a just perceptible decrease. During the transition from BR to PS the total lipid levels in Bank myna exhibited a steep fall to the lowest points of the cycle but the fall in these levels in Brahminy myna were less in magnitude. Thereafter lipid levels showed noticeable rise in both tissue of Bank myna and only that of the intestine in Brahminy myna. Another species-specific difference being lowering of lipid of the kidney of Brahminy myna throughout the cycle.

In case of Bank myna the TC and EC variations in the kidney as well as intestine exhibited similar patterns except for a remarkable departure in the case of intestinal

TC which showed a distinct decrease during BR and recovery during BR to PS.

The pattern of variations in TC and EC contents of the kidney and intestine of Brahminy myna were comparatively more consistent and parallel within themselves than the Bank myna; that is the obvious decrease in case of intestine of Bank myna was absent. In Brahminy myna the values in both tissues for both the parameters exhibited a gradually reducing trend from PR to BR to PS; the lowest values of annual cycle being recorded during the last mentioned phases. However, all these values started rising from PS to NB and rising in most of the cases above the PR level.

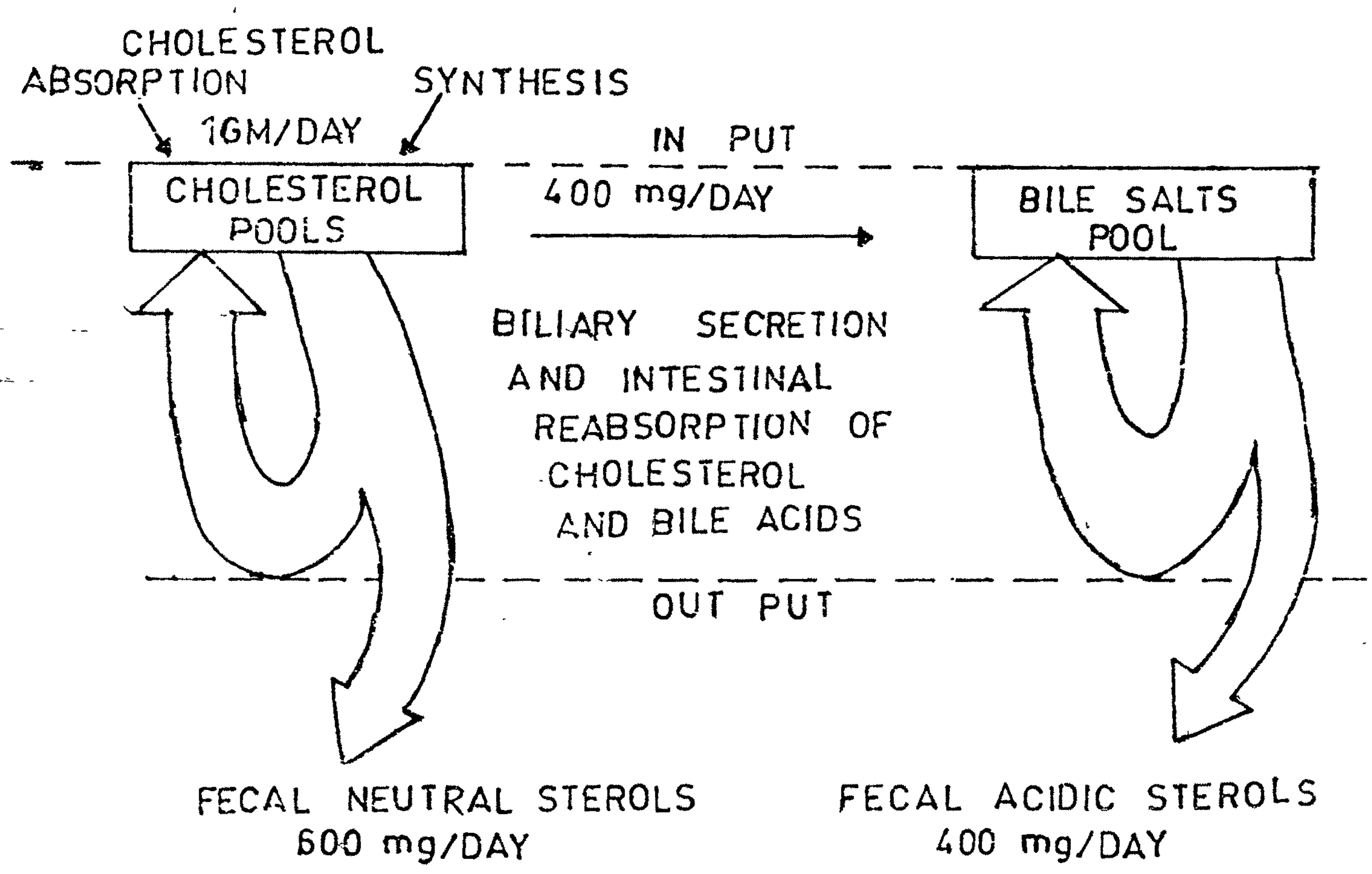
DISCUSSION

In case of majority of seasonally breeding birds, reproduction is restricted to a comparatively shorter but most favourable period of the year, when gonads are highly active in gametogenesis. Synthesis of steroid hormones utilizing cholesterol as precursor is a well established fact.

Anderson and Javit (1974) have reviewed inter-relationship between metabolism and bile acid synthesis and the reactions which take place in the liver and the intestine. Large amount of cholesterol is known to be secreted in bile, half of which is reabsorbed in the small intestine and remaining cholesterol undergoes structural alteration to coprostanol and other sterols found in faeces (Quintao, 1971). Cholesterol balance in man is given in the Fig. 3c merely from reference stand point.

The role of kidney in the excretion of steroid hormones as well as products of cholesterol is also a well established fact. Though enough histochemical studies on renal steroid dehydrogenases have been reported in several chordate species, only a few reports are available on G.I. tract (Baillie et al., 1966). Among birds, some of these enzymes were observed in the kidney of white-breasted water hen (Bhujle and Nadkarni, 1974) and kidney and intestine of pigeon (Kotak, 1979). Presence of some of these enzymes has been attributed to inter conversions between oestradiol and estron, and androstenedione and testosterone (Baillie et al., 1966). The reaction occurs all throughout the nephron but it is prominent in convoluted tubules. After studying dehydrogenase activities in extra gonadal tissues like liver, kidney and intestine, Ambadkar and Kotak (1976) reported that ilium plays a minor role in interconversion of steroid hormone, whereas Fellegiova et al. (1975) have reported through in vivo autoradiographic studies that injected testosterone is metabolized mainly in the G.I. tract.

Fig. 3c : Diagram showing enteró-hepatic circulation of cholesterol and bile salts in mammals.



FROM ANDERSON and JAVIT (1974)

Fig. 3c

Taking into consideration the above mentioned views, an attempt has been made during the course of present investigation to understand possible significance of changes taking place in the levels of AA, total lipids, TC and EC in kidney and intestine of seasonally breeding birds at different phases of reproductive cycles.

Notwithstanding the individual variations based on either the species, sex or phases of the reproductive cycle, the following overall generalizations emerge from the data presented here :-

i) AA levels were comparatively higher during PR and were at their minimum during the NB; more so in the Brahminy myna than in the Bank myna.

ii) In respect of the rest of the biochemical parameters there was a general agreement as far as the registration of minimal values for all parameters during PS and an increasing trend from PS to NB. In contrast with the trends in total lipid variation in Brahminy myna, kidney as well as intestine of the Bank myna exhibited minimum divergency of variations indicating much closer agreement.

According to the work of Roy and Guha (1958) and Ray Chaudhury and Chatterjee (1969) the liver of Bank myna is apparently the main site for AA synthesis. However, here it should be stated that, these authors did not take into consideration the phases of reproductive cycles while giving this opinion, but from the data presented here it seems that the kidney of not only the Bank myna but also that of Brahminy myna may be the possible complementary site of AA synthesis; at least during pre-breeding phases of the cycle. It is not unlikely that this may be happening under the influence rising titres of hormones concerned with reproduction. This seemingly occurs earlier during PR itself in male birds of both species whereas in female birds the influence begins to manifest itself during BR. By way of corollary, AA synthesizing activity seizes too, earlier in male birds, but that in females continues through BR to PS. It is therefore, possible that, in case of male birds lowering AA content not only in the intestine but also in the kidneys, probably points to its increasing release in general circulation in order to compliment increasing biosynthesis of sex steroids preparatory to active spermatogenesis. As opposed to this, such metabolic demand is more commonly known to occur in case of female birds actually during laying down of yolk material in breeding season. Complementary spurt in mobilization of cholesterol (TC as well as EC) should facilitate deposition of yolk. Additionally, as evident from the work of Nagorna-Stasiak _et_ _al_. (1986), apparently the intestinal rate of absorption of dietary AA also seemed to be transitorily influenced under these changing hormonal conditions to a noticeable extent during PR. It could be stated here from the field observations during the pre-breeding phase that the diet of both species of myna consisted of more of fruits and berries available in the post-spring season of the year providing a better source of dietary AA.

For the sake of better understanding about the role of TC and EC components, it was thought more convenient to consider the ratios of esterified cholesterol (EC) : total cholesterol (TC) rather than as separate entities. Following this line of understanding, it could be easily seen from the data presented here that in the case of female Bank myna the increase of intestinal total lipid content from PR to BR was accompanied by a significant rise in the ratio of EC : TC (from 0.5 to 0.9). A similar trend was true in the case of kidneys also. This was not the case in Brahminy myna. In the light of these facts it can be suggested that the role of intestine in Bank myna during PR to BR appears to be of importance as far as greater mucosal uptake and resynthesis of EC is concerned. Its subsequent incorporation into chylomicrons would lead to greater input for meeting the accelerating demand of the ovary for laying down of yolk material (BR). In Brahminy myna such a change is not apparent indicating that deposition of EC as well as TC fractions of yolk material occurred comparatively slowly and late.. This observation corroborates an early start of breeding activities and egg laying in case of Bank myna in contrast to the Brahminy myna, fact noted by Whistler as early as 1927. From the data it can be easily seen that, in contrast to overall decrease in the parameters during BR to PS, those of intestine and kidney of only male Brahminy mynas showed noticeably increased values of AA. In corroboration with this a highly significant drop in the EC : TC ratio - from 0.45 to 0.28 was also significant from BR to PS. Under these circumstances, it could be suggested that post-spermatogenic accumulation of cholesterol must be occuring at a comparatively earlier point during the BR to PS transition in the case of male Brahminy myna, hence much shorter breeding season in the male birds of this species. This seems to be the first reported evidence of early ending of breeding capacity in the case of Brahminy mynas. Applying similar line of discussion in respect of Bank myna it can be seen that this species has a comparatively wider spectrum of breeding activity, which is borne by the transition of EC : TC ratios from BR to PS suggestive of rising levels of post-nuptual testicular accumulation of cholesterol-positive lipids.

The species-specific differences were clearly noted in the case of these two species during different phases of reproduction in most of the parameters studied; indicating that both species have different metabolic demands at different phases of annual reproductive cycles.