Mycological Institute, Kew under the accession No. IMI 254637.

Colonies in agaro Czapekii 4.0 cm in diameter decem diebus 30 C crescentes, leviter elevatae radiantibus vel inaqualibus, primo albo-brunneae deivide flavae usque, secundum culturam. Capitula conidica sicutbreves serii rami emergentia, in mycelio intorta, in primo radiantia, deinde columaria plerumque conica. Conidiophorae plerumque $37.4 - 73.1\,\mu \times 3.4 - 5.1\,\mu$ brevis, enodes vesiculae ut lagenae formate in extreme partae, obconicus $8.5\,\mu$ in summ parte sola fertiles. Sterigmeta duo Seriata, primaria pauca $3.4 - 5.1\,\mu \times 3.4\,\mu$, secondaria $5.1 - 10\,\mu \times 3.4\,\mu$ saepe elongatis, septatis, parvis capitulis secundarris ferentibus. Conidia globose vel Subglobosa, inequaliter echinulata; comicliodorum massae obscura viridia, conidia $2.8 - 3.5\,\mu$ in dia. Nulla hulle cellula.

On the basis of synoptic key to *Aspergillus nidulans* group[1,2], taking into account the conidiophore length with other culture characteristics, the new fungus shows affinities with *Aspergillus unguis* (E-W. S G.) Thom & Raper, but differs from it, in the absence of typical spicular hyphae that arise from foot-cell and in funiculose growth in early stages due to abundant proliferating structures, bearing secondary conidial heads (figures 5, 6). Because of its stricking resemblance with *A. unquis* the new fungus has been named as *A. sub-unguis*.

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1. Christensen, M. and Raper, K. B., *Trans. Br. Mycol. Soc.*, 1978, **71**, 177.
2. Christensen, M. and States, J. S., *Mycologia*, 1982, **74**, 226.
3. Horticultural Colour Chart. The British Colour Council in collaboration with the Royal Horticultural Society. 1938.

## STUDIES ON THE DEVELOPING EMBRYONIC GONADS OF THE PIGEON, *COLUMBA LIVIA*

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Studies on the developing embryonic gonads in birds are restricted to that of chick, quail and duck. Gonadal tissue can be identified on the median surface of the Wolffian body as an elongation of the urogenital ridge in chick[1] by the fourth day of incubation. Earlier studies on the developing gonads of chick, quail and duck reveal that 6.5 day stage represents sex differentiation stage in chick, 5.5 day stage in the quail and 8-day stage in duck[1]. *In vivo* investigation by sex-hormone administration, castration experiments, *in vitro* culture of avian gonads and measurement of plasma steroids have shown that differentiation of gonads is under the influence of hormonal secretions of the gonads[2–8]. $\Delta^5$-3$\beta$-hydroxysteroid dehydrogenase ($\Delta^5$-3$\beta$-HSDH) has been localised in the embryonic gonads of chick, quail and pigeon[2,3,9–15]. In the present work, the development of the gonads in *Columba livia* has been studied from 3-day old embryo till 10 days to find out the age of the embryo at sex differentiation and to follow the various stages of the development of the testis or ovary. Further, an attempt is made to find out the relationship between ontogenetic steroidogenesis and sex differentiation in *C. livia* by histochemical localization of $\Delta^5$-3$\beta$-HSDH and glucose-6-phosphate dehydrogenase (G-6-PDH) activity in undifferentiated gonads till their differentiation into the testis or the ovary.

Fertilized eggs (3–10 day old) of pigeon were obtained from the pigeon colony maintained by the Zoology Department. The embryos were decapitated and the torso containing the vertebral column, adrenals, gonads and the renal tissue were fixed in Bouin's fluid and processed for histological studies. Some of the embryos from day 3 to day 10 were quickly frozen over dry ice and processed for the histochemical assay of $\Delta^5$-3$\beta$-HSDH and G-6-PDH enzymes as described earlier[16].

The present study reveals that the gonadal component can be detected in 3-day old embryos in *C. livia* and it is composed of the genital ridge and a few primordial germ cells (PGCs) (figure 1). The presence of PGCs along the dorsal mesentery at this stage indicates the probable extragonadal origin of PGCs as suggested earlier[4,17]. The structure of the gonads in *C. livia*

remains unchanged but for the increase in size of gonadal tissue and in the number of PGCs till the 6th day. The presence of mitotic figures in some of the PGCs suggests that the increase in their number in undifferentiated gonads is due to mitosis.

In embryos between day 6 and 7, there is a considerable increase in the size of the gonad, and it is differentiated into an outer cortex and an inner medulla (figure 2). Some of the embryos between day 7 and 8 showed an equally prominent cortex and medulla (figure 3) whereas in some of the embryos of the same age the gonads had a well-developed medulla and a thin cortex (figure 4). Further, in the embryos showing a prominent cortex in the gonad the germ cells predominate the cortical region, whereas in embryos showing prominent development of the medulla, the germ cells are mainly restricted to the medulla. When the germ cells are found in the cortex, the embryo develops into the female and the prominent presence of germ cells in medulla indicates its differentiation into testis. In all vertebrates, the cortex induces female differentiation and the medulla induces male differentiation of germ cells located within their range[18].

Some 9–10 day old embryos showed the gonads with distinct formation of medullary seminiferous cords suggesting their development into the testis, whereas in a few embryos of the corresponding age, gonads showed an increase in the size of the cortex and in the number of the germ cells. The absence of seminiferous cords in the medulla and the presence of germ cells in the cortex of the gonads of these embryos indicate that the embryonic gonad will develop into an ovary.

In birds, the morphological asymmetry of the gonads allows distinction of genetic sex of the embryo at the time of sex differentiation. In *C. livia*, in some of the embryos, the asymmetry of the gonads becomes evident by 9–10 days, the right gonad being smaller than the left. Further, the cortex of the right gonad is not so well-developed as that of the left gonad. These observations give additional indications that these embryos will develop into females with left functional ovary.

Table 1 shows the distribution of $\Delta^5$-3$\beta$-HSDH and G-6-PDH enzyme activities in the embryonic gonads of *C. livia*. The $\Delta^5$-3$\beta$-HSDH is an enzyme which plays a key role in the early stages of steroid biosynthesis. The localization of $\Delta^5$-3$\beta$-HSDH at the cellular levels in embryonic gonads of chick, quail and pigeon has been described[2,3,9,15]. In chick, $\Delta^5$-3$\beta$-HSDH activity has been reported in the genital ridge of 2.5 days[15], in undifferentiated gonads of 6.5 days[13,14], in the differentiated gonads of 8 days[11,12] and of 9 days[10].

**Table 1** *$\Delta^5$-3$\beta$-HSDH and G-6-PDH activity in the embryonic gonads of Columba livia*

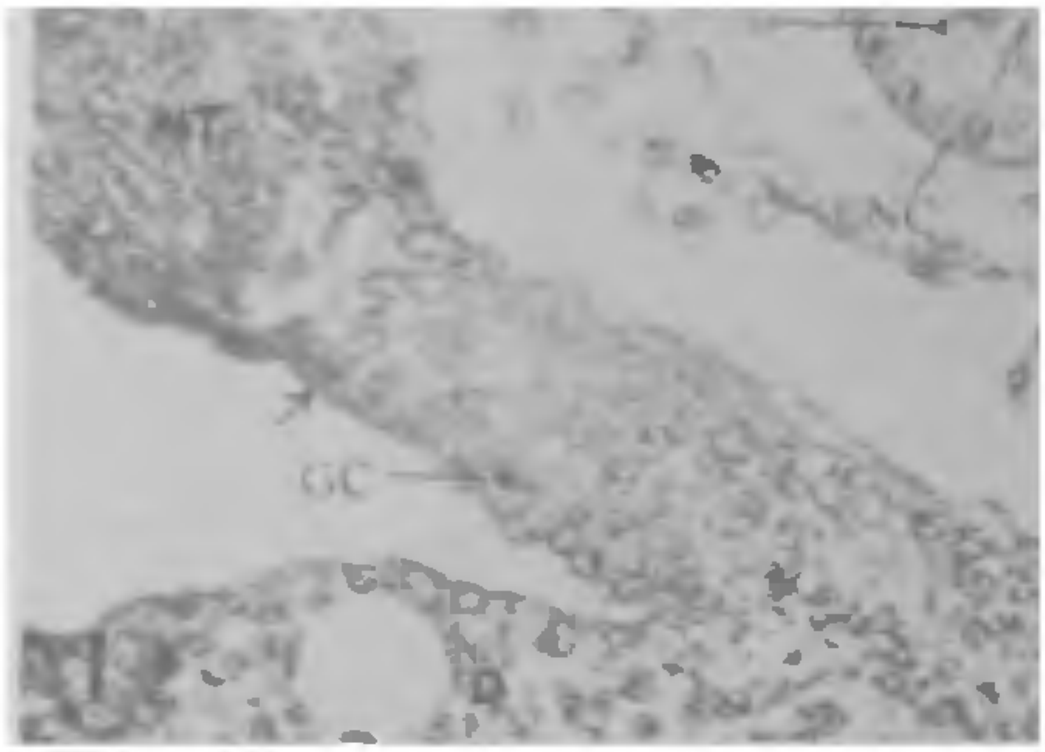
| Age of embryo (days) | Enzyme and substrates* | Gonads† Cortex | Gonads† Medulla |
|---|---|---|---|
| 6–7 (undifferentiated gonad) | $\Delta^5$-3$\beta$-*HSDH* | | |
| | 1. DHA | — | ± |
| | 2. Pregnenolone | — | ± |
| | *G-6-PDH* | — | + |
| 9–10 (Female) | $\Delta^5$-3$\beta$-*HSDH* | | |
| | 1. DHA | — | + |
| | 2. Pregnenolone | — | + + |
| | G-6-*PDH* | — | + + + |
| 9–10 (Male) | $\Delta^5$-3$\beta$-*HSDH* | | |
| | 1. DHA | — | ± |
| | 2. Pregnenolone | — | + |
| | G-6-*PDH* | — | + + |

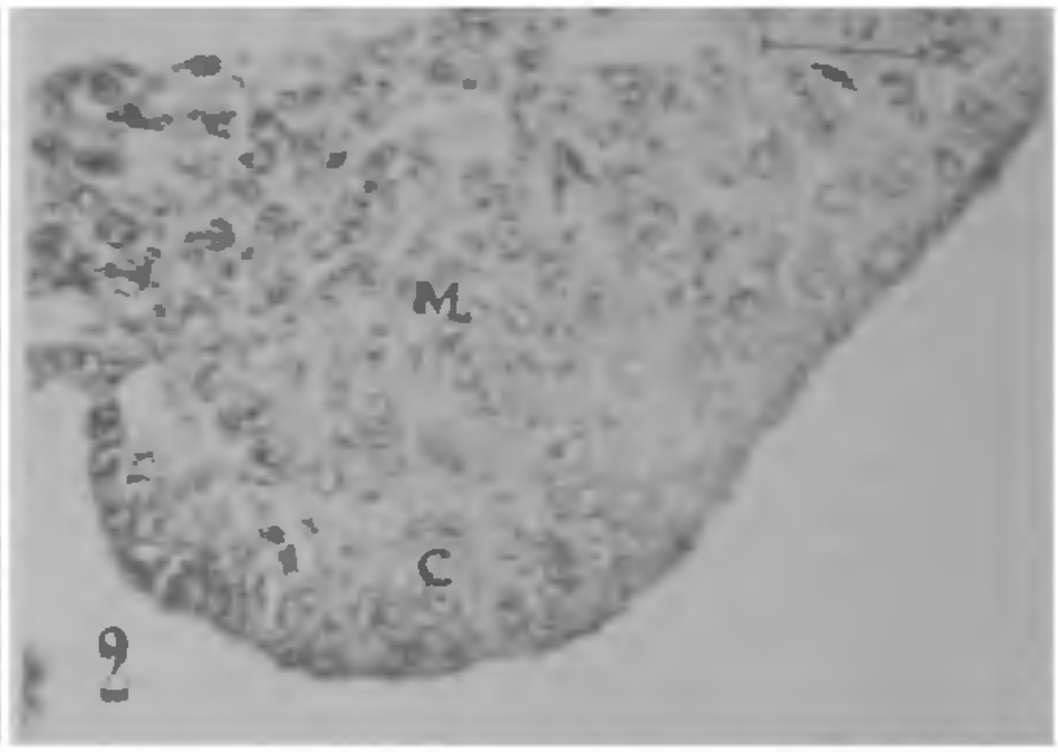
* Intensity of enzyme activity is graded from (−) to (+ + +); (−) denotes absence of activity and (+ + +) a maximal activity.

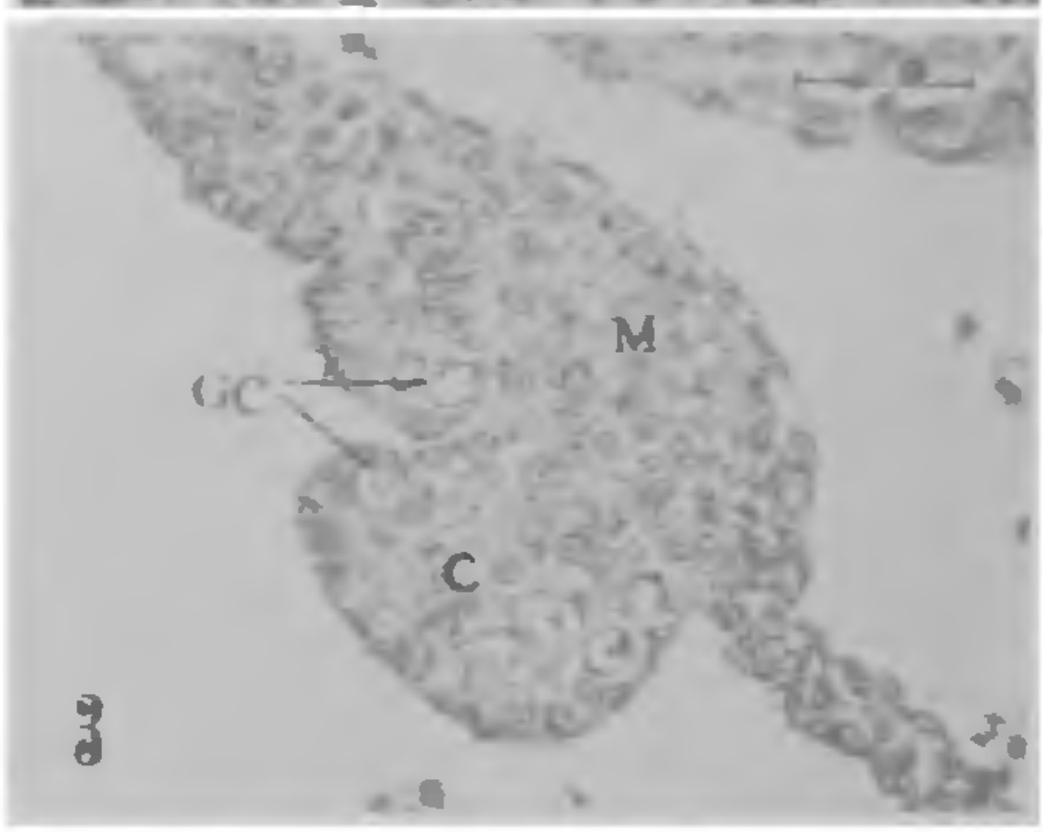
† Please note that enzyme activities were absent in the undifferentiated gonads of 3–5 day old embryos.

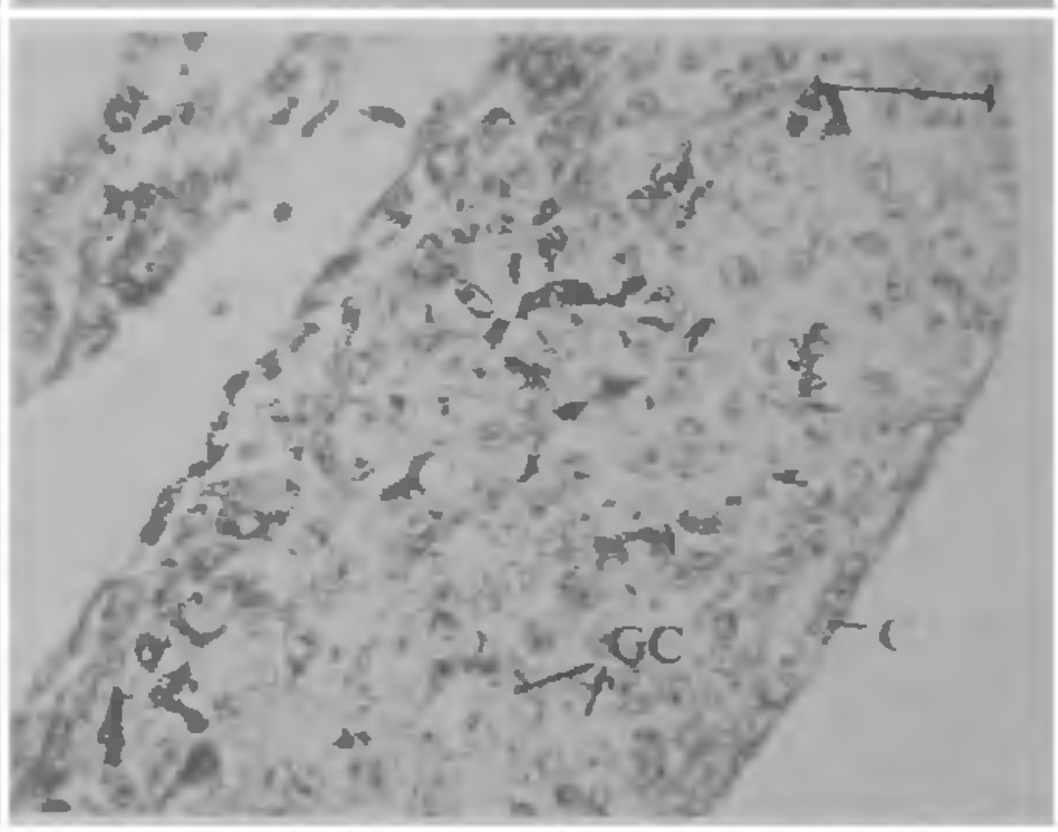
These studies indicate a disagreement about the stage at which $\Delta^5$-3$\beta$-HSDH activity appears in the embryonic gonads of the chick. Further, $\Delta^5$-3$\beta$-HSDH activity has been reported in the embryonic gonads of quail and pigeon only after the differentiation of the sex[9,14]. The presence of feable $\Delta^5$-3$\beta$-HSDH activity in the medulla of the undifferentiated gonads of 6-day old embryo of *C. livia* indicates its steroidogenic potential just prior to sex differentiation similar to that reported in chick[3]. The presence of $\Delta^5$-3$\beta$-HSDH activity in the medullary cell clusters (figure 5) in the embryonic ovary and the medullary cords (figure 6) of the embryonic testis of 9–10 days old embryos suggests that embryonic medulla forms an important site of steroidogenesis in the embryonic gonads in *C. livia*. It is pertinent to mention at this juncture that $\Delta^5$-3$\beta$-HSDH positive cells of the medulla of the female gonad seem to migrate to the cortex after the hatching of the young one and these cells probably contribute to the interstitial cells of the ovary[9].

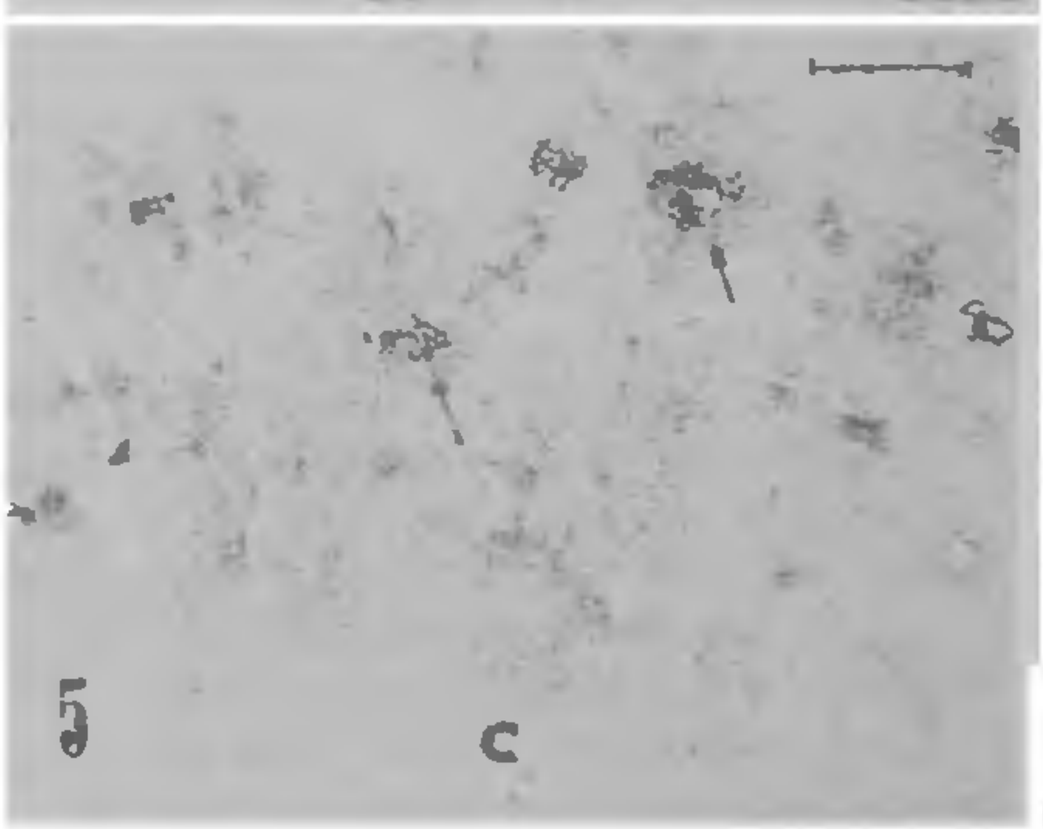
It has been reported in the chick and duck that embryonic ovary is more active in the production of steroids than the embryonic testis[5,7,9]. In the present study the greater intensity of $\Delta^5$-3$\beta$-HSDH activity in the medullary cells of the embryonic ovary than that of the embryonic testis in *C. livia* of 9–10 days suggests that the embryonic ovary might be more active in synthesizing steroids than the testis as reported in chick and duck[7].

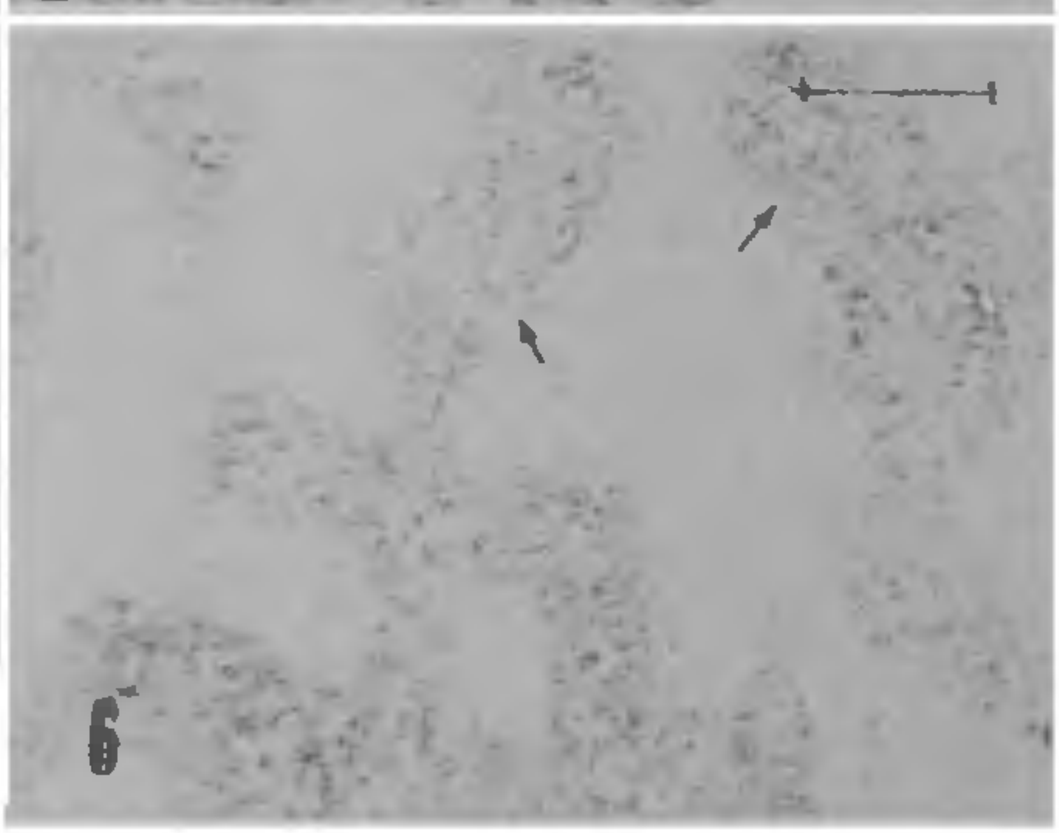



**Figures 1–6: 1.** T.S. of 3-day old embryo of *C. livia* showing a genital ridge (arrow). A primordial germ cell (GC) is seen embedded in the genital ridge. NC = nerve cord, MT = mesonephros, D = dorsal mesentery. **2.** T.S. of undifferentiated gonad of 7-day old embryo of *C. livia* showing the formation of cortex (C) and Medulla (M). **3.** T.S. of ovary of 9-day old embryo of *C. livia* showing a well-developed cortex (C) with prominent primordial germ cells (GC). M = medulla. **4.** T.S. of testis of 9-day old embryo of *C. livia* showing degenerated cortex (C) and formation of seminiferous cords (arrows) in the medulla. GC = primordial germ cells. **5.** $\Delta^5$-3$\beta$-HSDH activity in the clusters of medullary cells (arrows) in the ovary of 9-day old embryo of *C. livia*. **6.** $\Delta^5$3$\beta$-HSDH activity in the medullary cords (arrows) of the testis of 9-day old embryo of *C. livia* (scale line = 30 $\mu$m).

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1. Romanoff, A. L., *The avian embryo: Structure and function*, Macmillan, New York, 1960.
2. Haffen, K., *Am. Zool.*, 1975, **15**, 257.
3. Haffen, K., In *The ovary*, vol. 1 (eds) S. Zuckerman, and B. J. Weir, Academic Press, New York, 1977, p. 69.
4. Guichard, A., Cedard, L., Mignot, Th.-M. and Haffen, K., *Gen. Comp. Endocrinol.*, 1977, **32**, 255.
5. Guichard, A., Cedard, L., Mignot, Th.-M. and Haffen, K., *Gen. Comp. Endocrinol.*, 1979, **39**, 9.
6. Tanabe, Y., Nakamura, T., Fujioka, K. and Doi, O., *Gen. Comp. Endocrinol.*, 1979, **39**, 26.
7. Woods, J. E. and Brazzill, D. M., *Gen. Comp. Endocrinol.*, 1981, **44**, 37.
8. Tanabe, Y., Takashi, Y. and Nakamura, T., *Gen. Comp. Endocrinol.*, 1983, **49**, 144.
9. Bhujle, B. V., Nadkarni, V. B. and Rao, M. A., *Gen. Comp. Endocrinol.*, 1979, **38**, 153.
10. Boucek, R. H., Gyori, E. and Alvarez, R., *Gen. Comp. Endocrinol.*, 1966, **7**, 292.
11. Chieffi, G., Manalli, H., Botte, V. and Mastrolia, L., *Acta Embryol. Morphol. Exp.*, 1964, **7**, 89.
12. Narbaitz, R. and Kolodny, L., *Z. Zellforsch.*, 1964, **63**, 612.
13. Scheib, D. and Haffen, K., *Ann. Embryol. Morphol.*, 1968, **1**, 61.
14. Scheib, D. and Haffen, K., *Gen. Comp. Endocrinol.*, 1969, **12**, 586.
15. Woods, J. E. and Weeks, R. L., *Gen. Comp. Endocrinol.*, 1969, **13**, 242.
16. Bhujle, B. V. and Nadkarni, V. B., *Histochem. J.*, 1976, **8**, 691.
17. Kannankeril, J. V. and Domm, L. V., *Am. J. Anat.*, 1968, **123**, 131.
18. Witschi, E., In *The biochemistry of animal development*, Vol. II, (ed.), R. Weber, Academic Press, New York, 1967, p. 193.

## FLIGHT MUSCLE-GLYCOGEN OF SOME BUTTERFLIES (LEPIDOPTERA)

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GLYCOGEN has been shown to be present in the insect muscles by some workers in the past[1-7]. But all these workers have estimated this energy-yielding compound from the flight muscles as a whole, irrespective of their type. Because of this lacuna, the present study has been undertaken on 8 species of butterflies to ascertain the differences if any for glycogen storage in the longitudinal dorsal muscles (LDM) and tergosternal muscles (TSM).

Various species of butterflies were collected in the early hours of the day from the Botanical Garden of the Punjabi University. The butterflies were rested for an hour in a spacious cage, where flower pots were kept. The specimens were then dissected under stereoscopic binocular in physiological saline, so as to obtain the longitudinal dorsal and tergosternal muscles. The terminology used is based on the work of Snodgrass[8]. The muscle samples were blotted dry, weighed and proceeded with, to extract and estimate glycogen. For this purpose, the method of Heatly[9] was used while the estimations were done according to Montgomery[10].

The estimated amount of glycogen in the two different types of the flight muscles *i.e.* LDM and TSM of 8 species of butterflies is given in table 1, which reveals no uniform pattern of glycogen deposition. Glycogen in TSM ranges from 1.55 to 5.58 mg/g wet wt and in LDM from 1.48 to 5.32 mg/g wet wt in the different species used in the present study.

Sacktor[4] while studying the flight fuel of insects has commented in general that the species belonging to order Diptera and Hymenoptera, use carbohydrates as the main substrate for energy, but Zebe[11] has expressed that in other insects including Lepidoptera and Orthoptera, fats are used, even though glucose is available. The present findings on the 8 species of butterflies reflect that though the contents of glycogen are not very high in comparison to certain other groups of insects, yet the reserves are available in the flight muscles of these insects fairly in good amount, and this observation provides a good reason to believe that the lepidopterns (butterflies) use this compound also for energy production. This observation also strengthens and supports the views expressed by van Handel and Nayar[12], who have recently questioned the exclusive use of fat in Lepidoptera. These workers have de-