Utilization of Ascorbic Acid During Post-Embryonic Development of Chick Skeletal Muscle

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Summary. Ascorbic acid is utilized during the post-embryonic differentiation of skeletal muscle fibres in chick. While the fibres lose their heterogeneity with regard to ascorbic acid, they continue to exhibit differences in their metabolic rates in terms of the succinate dehydrogenase activity throughout life.

The importance of ascorbic acid (AA) in the maintenance of carbohydrate metabolism in the living cells, and also as an activator of a number of enzyme systems, has been known for a long time[2,3]. In the skeletal muscle fibres, which exhibit heterogeneity with regard to the carbohydrate vis-à-vis lipid metabolism, and the corresponding variations in the enzymic concentrations etc.[4], AA has been employed as a parameter for distinguishing the metabolically more active 'red' fibres from the 'white' type of muscle fibres, which have a relatively lower rate of metabolic activity[5]. Since the skeletal muscle fibres pass through a period of post-embryonic differentiation[6] from a common stock of morphologically and physiologically similar fibres[7] before attaining heterogeneity, it is logical to expect changes in the AA levels in the muscle fibres during this period of differentiation. The present investigation was undertaken to study histochemically, the variations in the AA concentrations of the muscle fibres during the post-embryonic development of a number of skeletal muscles in the chick. Succinate dehydrogenase (SDH) activity of the muscle fibres was used as an index of metabolic rates of the respective fibre types.

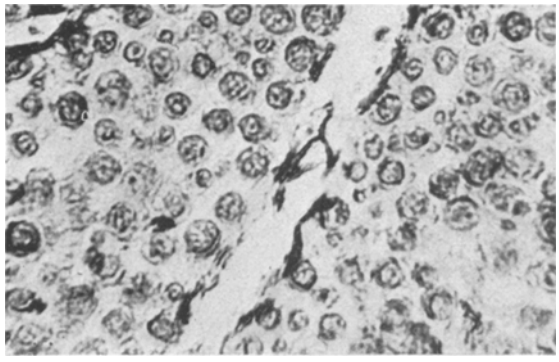

Fig. 1. T.S. of M. pectoralis at 1 day post-hatching. Note the uniform deposition of silver precipitate in the fibres. × 310.

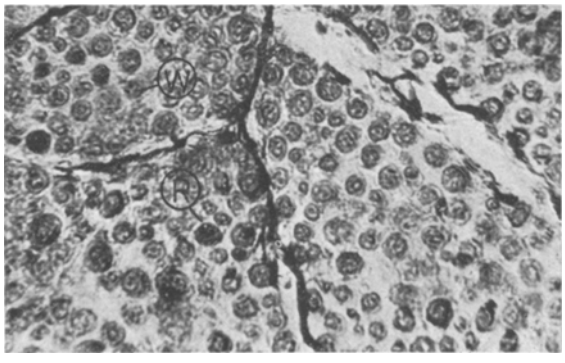

Fig. 2. T.S. of M. supracoracoideus at 3 days post-hatching. The 'red' fibres (R) show heavy AA concentrations compared to the 'white' fibres (W). × 200.

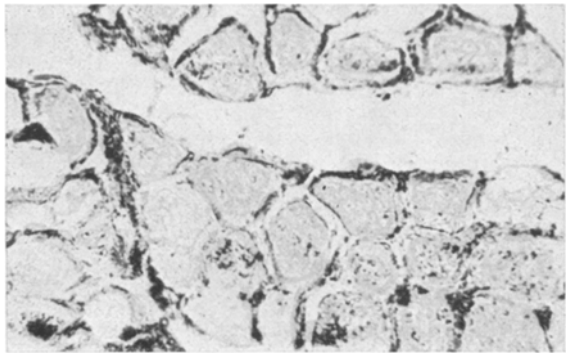

Fig. 3. T.S. of M. biceps at 30 days post-hatching. The fibres have lost heterogeneity with regard to their AA levels. × 300.

Materials and methods. 1-day-old chicks of White Leghorn variety were procured from the Government Poultry Farm at Chandigarh (India). These were maintained under suitable laboratory conditions and were sacrificed at 1, 3, 5, 7, 9, 30 days and the adult stages of life. 6 muscles – namely, the M. pectoralis, M. supracoracoideus, M. biceps and the M. gastrocnemius (pars externus, medius and internus) were dissected out and were subjected to alcoholic-acidic $AgNO_3$ for AA localization, by the method of Barnett and Bourne[8], with slight modification. Paraffin sections (12 µm) were mounted after clearing in xylene. The histochemical localization of SDH was made by the method of George and Talesara[9], using neo-tetrazolium chloride as the electron acceptor. Fresh frozen hand-cut sections were used.

Observations. On the 1st day of their post-embryonic development, all the fibres comprizing the muscles under investigation have a uniformly high AA content (Figure 1). At the 3 days stage, there is a general increase in the AA contents of the muscle and the two types of muscle fibres can be distinguished on the basis of difference in the intensity of the $AgNO_3$ reaction. The 'red' fibres show a heavy concentration of black granules in the middle, whereas the 'white' fibres appear brownish with a small amount of scattered granulation (Figure 2). AA is also present in appreciable quantities in the interfibrillar spaces and the interfascicular connective tissues. During the 5–7 days period, AA levels appear to decline and the granulation in the fibres becomes less distinct. The heterogeneity of muscle fibres on the basis of the $AgNO_3$

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reaction is lost after 30th day of post-embryonic life (Figure 3). The decline in the AA concentrations of the muscle fibres continues up to the adult stage of life when we observe negligible amounts of reduced $AgNO_3$ with a diffused distribution in the muscle fibres. The sarcolemmal reaction, however, is still distinctly observed.

The histochemical profile obtained with the localization of SDH activity in the muscle fibres during the early stages of development, appears identical to the one obtained with the $AgNO_3$ reaction. From a common stock of fibres at the 1st day post-hatching, distinct fibre types are differentiated at the 3–5 days period (Figure 4). The 'red' fibres have a high rate of SDH activity compared to the 'white' fibres which exhibit a low metabolic rate. This differentiation of muscle fibres on the basis of SDH activity is maintained throughout life.

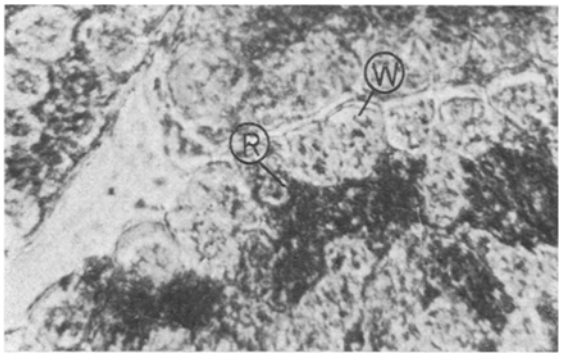

Fig. 4. T.S. of M. biceps (free hand section) at 5 days post-hatching showing the localization of the enzyme SDH. Heavy deposition of formazan in the 'red' fibres distinguishes them from the 'white' fibres. The heterogeneity is maintained throughout life in most of the muscles. ×450.

Discussion. The present investigation has revealed that the skeletal muscle fibres have higher AA concentrations during the early stages of their post-embryonic differentiation, and also confirms the view that the younger tissues have higher AA levels than the older ones[10,11]. The differentiation of fibre types from a basic stock of morphologically and physiologically similar fibres not only verifies the findings of earlier workers[6,7] but also is in conformity with our studies on the glycogen and lipid levels of the differentiating fibres[12]. The decline in the AA levels of the muscle fibres after 5–7 days post-hatching, points towards the importance of AA in the process of post-embryonic differentiation. There is thus a clear indication that AA is utilized during the post-embryonic differentiation of skeletal muscle fibres, and when this has been achieved, the muscle fibres lose their heterogeneity with regard to this metabolite. The muscle fibres, however, continue to exhibit heterogeneity in terms of the difference in their SDH activity, which has been widely accepted as a physiological parameter for distinguishing the muscle fibre types[13,14]. As such, the present observations call for further work before AA levels can be accepted as a parameter for studying heterogeneity in the adult skeletal muscle fibres. The continued presence of heavier concentrations of AA in the sarcolemmal and the extrafibrillar regions may be due to the proline-hydroxyproline conversion which is so very essential for the process of collagen synthesis[15].

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Tumor Incidence in Visna Virus Inoculated Mice[1]

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Summary. Mice (female Swiss albino) inoculated when newborn with Visna virus had tumors in 77% of cases when examined 8–12 months later. The tumors were mainly of the mammary carcinoma type. The tumor incidence in non-infected control animals was only 20%. In contrast, no increased incidence of tumors was observed among Visna virus-inoculated inbred mice (BALB/c, CBA and DBA) with low incidence of spontaneous mammary carcinoma.

Like the oncorna viruses, Visna virus contains an RNA-dependent DNA polymerase[2,3]. It has also been assumed that Visna virus might possess oncogenic properties. Transformation of mouse cells by Visna virus has been reported[4] and inoculation of Visna virus transformed cells into syngeneic suckling mice or irradiated young mice was associated with formation of tumors. Human cells of malignant astrocytoma infected with Visna virus produced low titers of infective virus and underwent morphological transformation[5]. The transformed cells contained Visna virus antigen.

However, tumor induction caused by the inoculation of cell-free Visna virus suspensions into animals has not been reported so far. The present study describes the development of tumors in mice inoculated as newborn with Visna virus.

Material and methods. Swiss albino mice (our own laboratory strain) were inoculated i.p. or s.c. when newborn with 0.1 ml of a Visna virus suspension (log ID_{50} between 6 and 6.5). The virus was produced and titrated in cultures of plexus choroideus cells of sheep. In total 39 Swiss albino females and 27 males were inoculated with Visna virus, while 25 females and 19 males were injected with saline buffer. In addition groups of newborn BALB/c, CBA and DBA were inoculated. All together 22 inoculated mice of each of these strains were studied. 16 to 18 of each strain were females. After the weanling period

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