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.

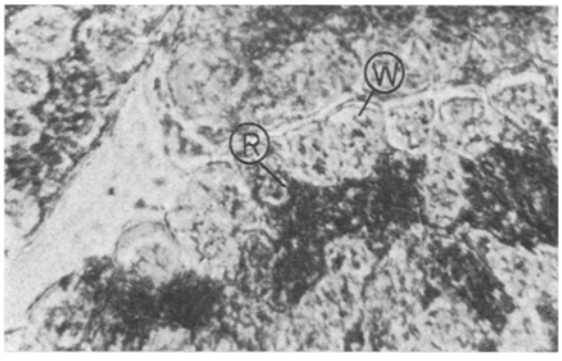

Fig. 4. T.S. of M. biceps (free hand section) at 5 days post-hatching showing the localization of the enzyme SDH. Heavy deposition of formazan in the 'red' fibres distinguishes them from the 'white' fibres. The heterogeneity is maintained throughout life in most of the muscles. ×450.

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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inoculated and non-inoculated mice were marked and kept together. Presence of tumors was investigated by macroscopical examination and palpation. Mice with tumors were autopsized, as were all mice 12 months after inoculation (Table).

Results and discussion. Tumors were observed after 8 months in both males and females. Table 1 summarizes the results. It is seen that tumor bearing animals were observed among the Visna virus infected Swiss albinos, as well as among the corresponding uninoculated or saline inoculated control animals. About 77% of the Visna virus infected female animals had tumors, while tumors were seen in 20% (p 0.001) of the female controls of Swiss albinos.

Incidence of tumors in Visna virus inoculated Swiss albino mice

Exp	Visna inoculated		Controls	
	Males	Females	Males	Females
1	0/13	8/14	0/12	1/12
2	3/14	22/25	1/7	4/13
1 + 2	3/27	30/39	1/19	5/25

Tumors of the mammary carcinoma type were observed in 39 mice and in 3 cases leukemias with enlarged spleens or livers were seen. Attempts to isolate Visna virus from tissues or blood of tumor-bearing animals by inoculation of plexus choroideus cells of sheep all failed.

About 10% of virus inoculated male Swiss albinos developed tumors, while tumors were found in 5% of male controls. The tumors seen in the males were cutaneous papillomas and leukemia.

In contrast, no increased incidence of tumors was observed among groups of 22 Visna virus-inoculated inbred BALB/c, CBA and DBA mice. Two of the virus-inoculated and two uninoculated mice died during the observation period and in one of the virus-inoculated animals, liver cysts were demonstrable.

Visna virus itself might not be directly responsible for the oncogenic effect observed. Increased incidence of mammary tumors was noted for the Swiss albinos but not for the mice of inbred strains with low incidence of spontaneously developed tumors. The above findings are consistent with preliminary experimental observations that inoculation of Visna virus in mice causes alterations in the immune system, i.e. functional changes in humoral and cell-mediated immunity. This will be reported in a following publication.

Differences in Susceptibility of Tissues to Revascularization Studied in Ectopic Implants

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Summary. The survival rate of implanted marrow is reduced when the tissue is transferred from one site to another within the first 24 hours but not more than 48 hours after the initial implantation. Splenic implants can be transferred at any time after implantation without affecting the survival rate. The observation suggests a difference in the sensitivity of the two tissues to the avascular period that they experience prior to initiation of angiogenesis.

Angiogenesis is a major limiting factor in growth, repair and regeneration of tissues. When the rate of tissue growth outpaces that of new vessel formation, circulatory insufficiency develops and results in necrosis or limitation of growth[2]. The autoimplantation of tissue pieces has served as a useful model to study the angiogenic potential of various normal tissues[2]. When tissues are so implanted, their normal circulation is severed and a successful take depends on new vessel growth with revascularization of the autoimplants. Implants of certain tissues, such as liver[2] and kidney[3] are not capable of eliciting revascularization; necrosis, typically of the coagulative type, supervenes. Implants of skin and bone marrow, on the other hand, show a great potential for angiogenesis[2]. These tissues are revascularized within 24 h after implantation ensuring a successful take in almost every experiment.

We have previously reported that spleen implants, although incapable of eliciting rapid angiogenesis, survive in ectopic sites[4], suggesting that the vulnerability of various tissues to the initial avascular period may also differ. In the present study an attempt was made to delay the revascularization of bone marrow and spleen implants to determine the vulnerability of these tissues to an initial avascular period. This was done by reimplanting the tissues in a new site at various intervals after initial implantation.

Materials and methods. Male Wistar rats (200–300 g) were used and anesthesia was given by i.p. injection of pentobarbital. The methods for ectopic implantation of marrow and spleen have previously been described[2,4]. Briefly, the knee joint was exposed and an opening made in the shaft of the femur, through its articular surface, using a low speed dental drill. A polyethylene tube (No 160) was then inserted into the shaft, the free end clamped and the tube then withdrawn. The marrow tissue, now filling the tube was expelled and implanted into a pocket made in the subcutaneous tissue of the abdomen through a midline incision. Fragments of splenic tissue 5 to 8 mm in diameter, were similarly implanted after the animals were splenectomized.

At various intervals the implants were removed and reimplanted, subcutaneously, on the opposite side of the midline abdominal incision. 5 or 6 weeks after the initial implantation the implants were studied histologically, and sequential studies of implants were made when indicated. The criterion of a successful take was the formation of normal-appearing splenic tissue or bone marrow nodule.

[1] The work was supported by a grant from NIH. The author is recipient of a Career Research Development Award from NIAMDD.

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