Time-dependent Inhibition of Myogenesis by Actinomycin D

MUSCLE owes its morphological and biochemical identity to proteins such as actin, myosin, tropomyosin, etc.,[1] and it is reasonable to suspect that these molecules are somehow represented in the genome. Until rather recently, it was generally believed that all new proteins are manufactured by freshly made messenger RNA's of exceedingly short half-lives. However, recently it has become rather evident that there are at least a few systems in which genetic messages are sent many generations before they are used[2]. Is this the case with myogenesis, or is the necessary information for muscle differentiation sent shortly before it is called into play?

Actinomycin *D* seemed to provide an experimental approach to the question. The antibiotic complexes with DNA and thereby prevents synthesis of RNA. At the same time, already-formed templates continue in the manufacture of proteins and DNA replication occurs[3]. The myogenic system selected for investigation was regenerating mouse skeletal muscle. This choice was dictated primarily by the fact that there is a great deal of synchrony attending the reaction: during the second to third day after muscle injury a class of highly proliferative mononucleated cells appears in the wound[4,5], and on the fourth day newly developing muscle fibres are in abundance. If the mononucleated cells are arrested by colchicine new muscle fibres fail to appear on schedule[5]. Moreover, if the mononucleated cells incorporate tritiated thymidine, differentiating muscle fibre nuclei show radioactivity after autoradiography[6,7]. Thus, it would seem that in the 3–4-day interval after wounding many presumptive muscle cells are achieving the differentiated state.

Transections were inflicted in tibialis anterior muscle of female Swiss-Webster mice, weighing 25–30 g, under light methoxyflurane anæsthesia. Animals received a single intraperitoneal dose of 0·99 micrograms of actinomycin *D* (kindly donated by Merck and Co.) at 24, 48, 72 or 100 h after transection. Tissues were fixed for histological investigation five days after wounding. Day five was chosen because at this time there normally are scores of regenerating fibres in each section through the wound[4–6].

Large numbers of typical five-day regenerating myotubes were encountered in all sections of the 24, 48 and 100 h series (Fig. 1). In five of five specimens given actinomycin at 72 h, regeneration was severely depressed (Fig. 2) and was represented by only an occasional, single, immature muscle fibre. Although few in numbers, these fibres were normal morphologically.

The findings indicate that myogenesis depends on a new round of RNA synthesis (presumably messenger) in the interval immediately preceding the histological advent of muscle as a tissue. This, in turn, suggests that muscle is specifically represented in the genome and that the information for making it is communicated shortly before it is used. Along this same line, it may be noted that muscle regeneration can be prevented by X-irradiation, but sensitivity to the effective dose is lost somewhere on the third day; that is, after the genetic messages seem to have been sent[8].

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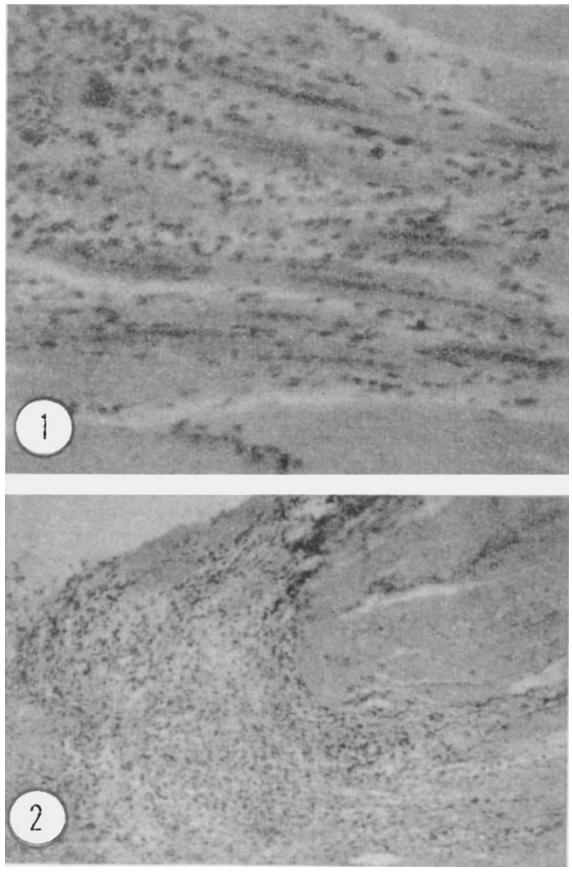

Fig. 1. Newly differentiating muscle fibres in five-day wound from animal injected with actinomycin *D* 24 h following muscle transection (× 200).

Fig. 2. Five-day wound coagulum from animal injected with actinomycin *D* 72 h after muscle transection. This representative field is devoid of newly differentiating muscle fibres. In normal regeneration and in cases injected with actinomycin *D* at 24,48 and 100 h such sections exhibited scores of differentiating muscle fibres. (× 100)

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Occurrence of Homoserine in the Liver of Ethionine- or Methionine-treated Rats

INTRAPERITONEAL administration of L-methionine[1] as well as incorporation of DL-methionine[2] and DL-ethionine[3] in the purified diets of rats resulted in increased activity of rat liver cystathionase ('soluble' cysteine desulphydrase). Recently, I have also observed[4] that one injection of L-ethionine likewise caused, in 4 h, significant increase in the activity of the enzyme in rat liver. In an effort to determine the chemical basis for the effects of these amino-acids, analyses of the free amino-acids of the liver of control (non-treated) as well as of ethionine and methionine-treated rats were carried out.

For this purpose, male Wistar rats, weighing 90–100 g, were fed for 7 days a purified diet previously described[2], containing 18 per cent casein. The control animals were killed without any treatment; the ethionine- and methionine-treated animals were injected respectively with 54 mg L-ethionine and 48 mg L-methionine per 100 g body weight, by intraperitoneal route, 4 h before death. Another group was fed the purified diet containing 1·5 per cent DL-ethionine for a week. Free amino-acids were extracted from representative samples of 1 g of liver according to the procedure described by Awapara[5]. Chromatographic analyses of the extracts were performed on Whatman paper[4] (descending method), solvents: butanol/acetic acid/water (50:25:25) and phenol/water (80:20), on thin-layer silica gel (ascending method) using the same solvents, and in a Technicon autoanalyser after passing the extract through