Stimulation of the alternative oxidase activity by GMP was found to be highest when the cytochromic pathway was inhibited by 1 mM KCN (fig.). When NADH was used, the stimulation was about 500% (fig., a) while the stimulatory effect of GMP on the succinate oxidase activity was only about 300% (fig., b). Both nucleotide activations were sharply inhibited by 2 mM SHAM or salicyl hydroxamic acid, a specific inhibitor of the mitochondrial cyanide-insensitive oxidase[12]. GMP was totally without effect on the oxygen consumption of mitochondria isolated from untreated conidia both in the presence of succinate (fig., c) or NADH as respiratory substrates. AMP, ADP, ATP and GTP were also tested and the highest stimulation (about 100%) was obtained with AMP.

The total activity of the alternative oxidase in mitochondria of heat-shocked conidia was assayed in the presence of 1 mM KCN and 1 mM GMP. Maximum inhibition of the alternative oxidase activity was in the range of 60–70% when 2 mM SHAM was used. KCN and SHAM together inhibited 90% of the oxidase activities. The residual respiratory activity is of unknown origin.

To germinate, the heat-shocked conidia have to be shifted-down to 25°C, at which temperature they efficiently recover an efficient ATP-producing cyanide-sensitive pathway[5].

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2 Present address: Département de Microbiologie, CMU, 9 ave. Champel, CH-1211 Geneva, Switzerland.
3 To whom reprint requests should be addressed.

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Developmental forms of human skeletal muscle AMP-deaminase

K. Kaletha, J. Spychala and G. Nowak

Department of Biochemistry, Medical School, ul. Debinki 1, PL-80-211 Gdansk (Poland), 22 July 1986

Summary. Chromatography on phosphocellulose revealed the existence of two well-separable forms of skeletal muscle AMP-deaminase in the tissue extracts of 11- and 16-week-old human fetuses. One of these forms elutes from the column at the same salt concentration as the muscle isozyme found in the skeletal muscle extract from adult man, and seems to have similar kinetic properties. The second form, which was found only in vestigial amounts in adult human tissue extract, represents different kinetic properties and seems to be a form characteristic for the fetal period of ontogenesis.
Key words. AMP-deaminase.

AMP-deaminase (E.C. 3.5.4.6; AMP-aminohydrolase) catalyzes irreversible hydrolytic deamination of 5'-adenosine monophosphate. The enzyme is widespread in animal tissues, although a considerably higher concentration is found in skeletal muscle than in other tissues, including heart and smooth muscle[3]. The enzyme of skeletal muscle is closely associated with myosin[4], participates in the purine nucleotide cycle[5], and catalyzes the reaction which is the major source of ammonia in the working muscle[6]. The total amount of AMP-deaminase in skeletal muscle varies during the course of development[7,8] and the changes in immunological, chromatographic and kinetic properties of the enzyme observed during this process indicate a developmental shift in the isozymic pattern[9–11].

Recently Fishbein et al.[12], using a new histochemical method, reported that the skeletal muscle of some patients is practically devoid of AMP-deaminase activity. The enzyme deficiency has been found in about 1% of muscle biopsies, with approximately equal frequency in males and females. All negative histochemical stains showed less than 5% of normal specific activity of the enzyme[13]. Sabina et al.[14] have found recently, that AMP-deaminase deficiency reduces the entry of adenine nucleotides into the purine nucleotide cycle during exercise, resulting in disruption of this cycle, and leading to muscular dysfunction. The deficiency appears to occur in two forms[15].

Table 1. AMP-deaminase activity in human fetal and adult human skeletal muscle extracts

Source of the enzyme	Activity (units*/per mg of protein)
11-week-old fetus (5)	0.03 ± 0.01
16-week-old fetus (1)	0.06
Adult man (1)	0.16

* One unit of the enzyme activity was defined as the amount of enzyme which catalyzes the formation of 1 μmole of ammonia per minute at 1 mM substrate concentration. Data are means from the number of determinations given in parentheses.

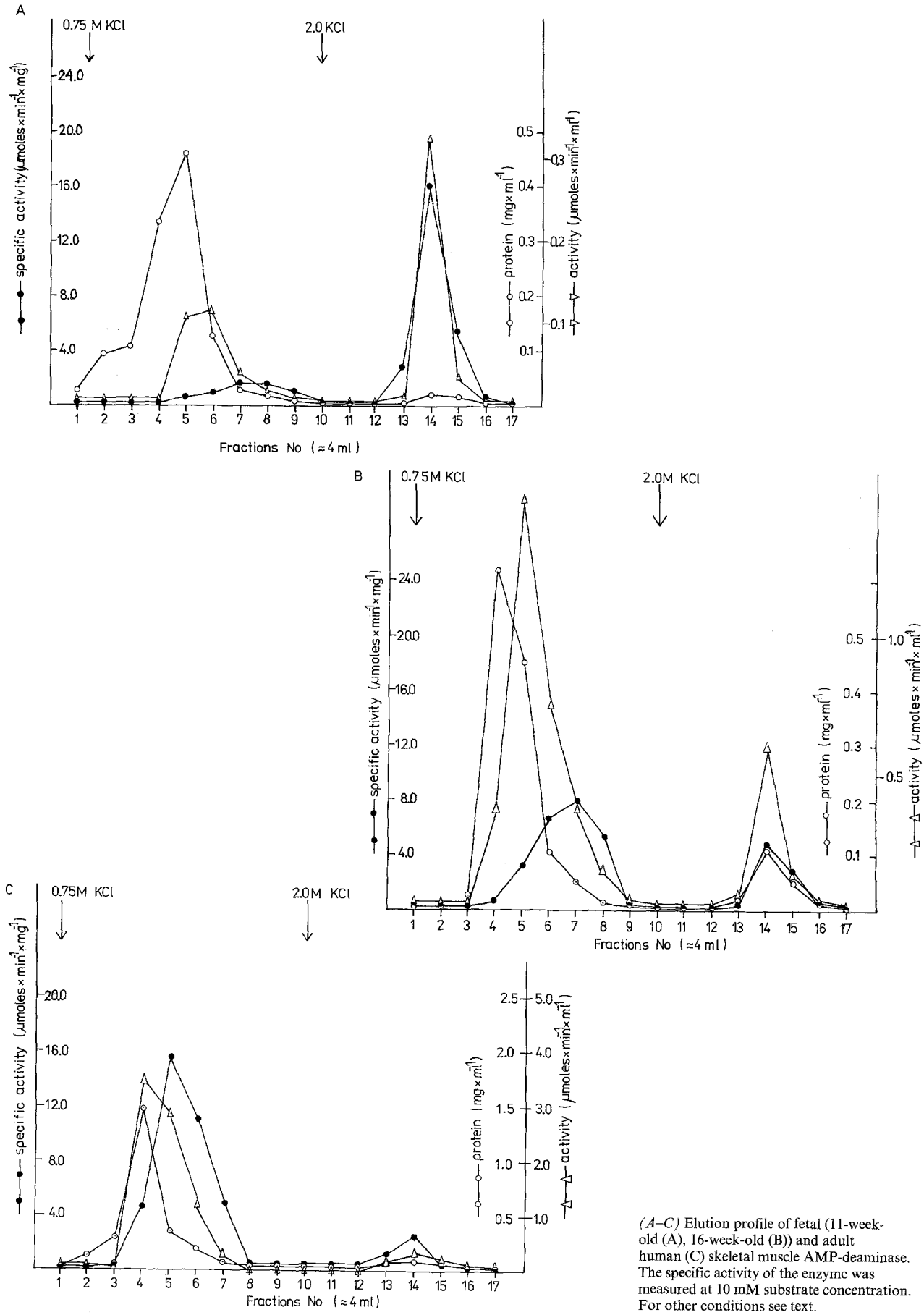
The primary form seems to be inherited as a complete gene block in an autosomal-recessive pattern. This form of deficiency is usually not symptomatic until middle or adult age, when muscle cramping and exercise intolerance develop. The patients have normal levels of AMP-deaminase in other tissues. Since the gene defect is not rare, in some cases it is associated with other neuromuscular disease.

The secondary, carrier form is quite common (10% in the muscle biopsy specimen population) and in this form of deficiency, the residual AMP-deaminase level is considerably higher, although clearly still deficient. Other muscle enzymes are also depleted, although not as severely.

Even in the primary form of AMP-deaminase deficiency one could find a trace of residual activity in the muscle tissue. In Fishbein's opinion such activity may represent a fetal muscle isozyme, or a blood-cell isozyme contaminating the muscle biopsy.

In this paper data are presented which indicate a developmental shift in the isozymic pattern of AMP-deaminase in human skeletal muscle.

Materials and methods. Material: Samples of human fetal leg muscle at the ages of 11 and 16 weeks of gestation were obtained from the Obstetric and Gynaecological Department of the Academic Medical School in Gdansk. Samples of 11-week-old hu-



(A–C) Elution profile of fetal (11-week-old (A), 16-week-old (B)) and adult human (C) skeletal muscle AMP-deaminase. The specific activity of the enzyme was measured at 10 mM substrate concentration. For other conditions see text.

Table 2. Comparison of the kinetic properties of two AMP-deaminase molecular forms purified from the muscle extract of 11-week-old human fetus

Molecular form of the enzyme	$S_{0.5}$ (mM)	v(5 mM/ 0.5 mM)	n_H
'Fetal' (eluted with 2.0 M KCl)	2.8 (0.3)	4.8 (0.9)	1.2
'Adult' (eluted with 0.75 M KCl)	0.7 (0.1)	1.8 (0.3)	1.0
Human skeletal muscle enzyme*	0.9[a] 0.6[b]		

* Muscle tissue samples taken during autopsy; [a] Kaletha et al.[22]; [b] Ogasawara et al.[23]. The values in the brackets represent SD.

man fetal muscle tissue were pooled for further purification, while that of the 16-week-old human fetus was processed individually. All fetal specimens were received and processed within 2–4 h. The sample of adult human thigh muscle (obtained from the City Hospital in Starogard Gdanski), taken in the course of surgery, was frozen and used after several days.

Enzyme purification: After removing the skin and connective tissue, the samples (weighing 3–8 g) were wiped free of adhering blood residues, washed, and homogenized in 15–20 vol (v/w) of extraction buffer. AMP-deaminase was isolated by chromatography on phosphocellulose, essentially according to the procedure of Smiley et al.[16]. The enzyme adsorbed on the column was eluted stepwise, with successive washing of the phosphocellulose with 0.75 M and 2.0 M KCl.

Each of five chromatographic separations, which were performed with 11-week-old fetal skeletal muscle extracts, gave well-reproducible results, and the activity distribution between the peaks was found to vary only insignificantly from separation to separation.

The most active fractions of 11-week-old human fetal muscles extract were collected, concentrated by ultrafiltration in a Schleicher-Schuell device, and used in the subsequent studies.

Enzyme assay: The incubation medium in the final volume of 0.5 ml contained 0.1 M potassium-succinate buffer (pH = 6.5), 100 mM potassium chloride and eight different concentrations of the substrate (in the range $(0.3 - 20.0) \times 10^{-3}$ M). After equilibration of the medium at 30 °C, 50 µl of the enzyme solution was added to start the reaction. The reaction was carried out for 20 min and the initial velocity of the reaction was determined from the mean ammonia amount liberated in two parallel incubations. No symptoms of enzyme denaturation were observed, as judged from the proportionality of the ammonia liberation versus time for the period up to 20 min. The phenol-hypochlorite method of Chaney and Marbach[17] was used for the estimation of ammonia production. Protein was determined with Coomassie-Brillant Blue[18].

The method based on linearization of the Hill equation, described by Endrenyi et al.[19] was used to calculate the kinetic parameters of the reaction.

Reagents: 5'-AMP (from equine muscle, type V) as well as 2-deoxyadenosine 5'-monophosphate (dAMP), adenosine 5'-diphosphoribose (ADPR), adenosine 5'-monophosphoramidate (AMP-NH_2), adenosine 2'-monophosphoric acid (2'-AMP) and adenosine 3'-monophosphoric acid (3'-AMP) were supplied by Sigma. Cellulose phosphate was from Whatman. All other chemicals were from Polskie Odczynniki Chemiczne.

Results and discussion. The activities of AMP-deaminase in the extracts of 11- and 16-week-old human fetuses, as well as in adult human skeletal muscle, are summarized in table 1. It may be seen from this table that the activity of AMP-deaminase found in 11-week-old human fetal extract was only about 20% of that in adult muscle, but it increased two-fold during the period between 11- and 16-weeks of gestation.

The elution profile of the enzyme isolated from the leg muscle of 11- and 16-week-old human fetuses and from the leg muscle of adult man is shown in the figure. As can be seen from this figure, the extracts obtained from both fetal and adult human skeletal muscle contain two well-separable peaks of AMP-deaminase activity, indicating the presence of two different molecular forms of the enzyme. In contrast to the adult, human muscle where the form eluted with 0.75 M KCl ('adult' form) represented the greater part of the total AMP-deaminase activity, the main enzyme form present in the muscle extract of the 11-week-old human fetus was that eluted with 2.0 M KCl ('fetal' form). The shift in the isozymic pattern seems to be rather quick at this stage of ontogenesis, as the activity of the 'adult' form of the enzyme is quite abundant already in the 16-week-old fetal muscle extract.

The two molecular forms have been found to differ distinctly in their stability. In contrast to the 'adult' form, which was quite stable and did not lose more than 5–10% of its initial activity during one day of storage in the refrigerator, the 'fetal' form of AMP-deaminase lost as much as 40–60% of its activity under the same conditions.

The two molecular forms of 11-week-old human fetus muscle AMP-deaminase differ from each other also in their kinetic properties. Although both displayed a similar, hyperbolic type of kinetics (not shown here), they did, however, manifest distinctly different half saturation constant ($S_{0.5}$) values. It may be seen from table 2 that the $S_{0.5}$ value of the 'fetal' form of the enzyme is about four times higher than that calculated for the 'adult' form. Also, the ratio of the reaction velocity measured at 5 mM substrate concentration, to that measured at 0.5 mM, is considerably higher for the 'fetal' form of 11-week-old human fetus skeletal muscle AMP-deaminase.

As shown in table 3, the two molecular forms of AMP-deaminase in 11-week-old human fetal skeletal muscle differ also in their substrate specificity. In general, the 'fetal' form of AMP-deaminase manifests a lower substrate specificity. There is also a difference in the ratio of dAMP/AMP deamination.

All these data indicate that, similarly to the chicken skeletal muscle enzyme[9–11], the expression pattern of human skeletal muscle AMP-deaminase also changes during ontogenetic development.

The lack of sufficient amounts of tissue material prevented us from performing a kinetic characterization of AMP-deaminase forms from more mature tissue (16-week-old fetus and adult human). One may assume, however, that the kinetic properties are relatively unchanging, and that the main factors undergoing change during ontogenetic development are the total enzyme concentration and the activity distribution between 'adult' and 'fetal' forms of the enzyme.

Considering the low adenylic acid concentration which normally exists in skeletal muscle[20], the effectiveness of the catalysis performed by the 'fetal' form of the enzyme in the early stage of ontogenesis, when probably only this form is present in the tissue, seems to be rather low. On the other hand, the distinct increase of the activity of the 'adult' form of the enzyme, observed between 11 and 16 weeks of gestation, indicates that AMP-deaminase begins to play an increasing role in the muscle metabolism at this period of fetal development.

It is highly probable that the 'fetal' form of the enzyme is that which is responsible for the remnant activity observed in the primary type of AMP-deaminase deficiency, in which a gene block would concern the expression of the 'adult' form of the enzyme.

Table 3. Deamination of some analogues of 5'-AMP by two molecular forms of 11-week-old human fetal skeletal muscle AMP-deaminase

Substrate (1 mM)	Relative velocity (%) 'Adult' form	'Fetal' form
5'-AMP (control)	100	100
2'-AMP	0.7	2.1
3'-AMP	0.6	2.2
ADPR	1.0	4.1
AMP-NH_2	1.4	0.2
dAMP/AMP*	17.0	8.8

* $\frac{\text{Rate of ammonia production with 10 mM dAMP}}{\text{Rate of ammonia production with 10 mM AMP}} \times 100.$

1 The studies described in this report were performed on dead aborted human fetuses. The abortion did not have any connection with the protocol of the study[21].
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Transdifferentiation of larval *Xenopus laevis* iris implanted into the amputated hindlimb[1]

C. Cioni, S. Filoni[2], L. Bosco, C. Aquila and S. Bernardini

Dipartimento di Biologia animale e dell'uomo, Università di Roma 'La Sapienza', via A. Borelli 50, I-00161 Roma (Italy), and Dipartimento di Biologia, Università di Roma 'Tor Vergata', Via Orazio Raimondo, I-00173 Roma (Italy), 9 July 1986

Summary. Fragments of larval *Xenopus laevis* iris, autoplastically implanted into the stump of the amputated hindlimb, transdifferentiated into neural retina. However, when such iris fragments were implanted into the caudal fin, no transdifferentiative process was observed.
Key words. Transdifferentiation; dorsal iris; larval *Xenopus*; amputated limb.

Lens regeneration from the outer cornea in larval *Xenopus laevis* depends on the presence of ocular factor(s), probably proteic in nature, coming from the neural retina[3-6]. However, it was demonstrated that the neural retina is not the only source of stimulus for transdifferentiation of corneal cells into lens, since promoting factors of lens-forming transformations of the outer cornea are also produced by several larval tissues. In particular, the outer cornea also responds to stimuli coming from regenerating limb, limb bud, limb blastema, tentacle blastema and spinal ganglia[5,7-9]. These data have been explained by assuming that a neurotrophic factor, produced by the ganglion cells and also by dedifferentiated cells of buds and blastema, is responsible for promoting the lens-forming transformations of the outer cornea[8,9].

In contrast to the outer cornea, iris epithelial cells of lentectomized *Xenopus laevis* larvae do not show any lens-forming transformation capacity even when this tissue has been injured in order to stimulate latent potentialities[10]. However, we recently demonstrated that iris fragments from *Xenopus laevis* larvae implanted in the vitreous chamber of lentectomized eyes can transdifferentiate into neural retina under the influence of promoting factors present in this eye territory[11]. Other investigations indicated that dorsal iris epithelial cells from adult frogs (*Rana temporaria*) show different transdifferentiative capacities under different experimental conditions[12]. The aim of the present work was to test the influence of the amputated hindlimb environment on transdifferentiative capacities of the dorsal iris from larval *Xenopus laevis*.

Materials and methods. A total of 55 *Xenopus laevis* larvae at stage 54-55 (according to Nieuwkoop and Faber[13]), anesthetized with MS 222 Sandoz at a concentration of 1:5000 in full strength Holtfreter's solution, were operated on. The animals were then gradually transferred into tap water and reared until the 20th day after the operation. The sacrificed animals were processed for O. M. (fixed in Bouin's solution, embedded in paraffin, cross-sectioned at 7 µm and stained with hematoxylin-eosin) or E. M. (fixed in 2% paraformaldehyde, 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, postfixed in OsO_4, dehydrated through an ethanol series, stained 'en bloc' with uranyl acetate and embedded in Spurr's resin). Thin sections were stained with lead citrate and observed on AEI 801. Two types of experiments were carried out: Experiment 1: Autoplastic implant of dorsal iris into the amputated hindlimb (fig. 1a). Experiment 2: Autoplastic implant of dorsal iris into the caudal fin (fig. 1b).

Results and discussion. The results are shown in the table. The data obtained in experiment 1 show that dorsal iris fragments implanted into the limb stump transdifferentiate into neural retina in 52% of cases examined, while 19% of cases undergo only a partial depigmentation of iris epithelial cells. The newly-formed neural retina has a typical stratification, with clearly identifiable photoreceptors (figs 2, 3, 4).

Summary of the results of experiments 1 (autoplastic implant of dorsal iris into the amputated hindlimb) and 2 (autoplastic implant of dorsal iris into the caudal fin)

Experiment	N° of cases operated*	N° of cases dead/ discarded	N° of cases examined	N° of cases with partial transformation	N° of cases transdifferentiated into retina
1	30	9	21	4	11
2	25	3	22	–	–

* Moreover, in both experiments five additional larvae were operated on and fixed immediately after the operation to serve as controls of the operative procedure.

The data obtained in experiment 2 show that the dorsal iris fragments implanted into the caudal fin undergo various degrees of regression and a partial depigmentation.