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Vitamin A-Mediated Homeotic Transformation of Tail to Limbs, Limb Suppression and Abnormal Tail Regeneration in the Indian Jumping Frog *Polypedates maculatus*

(anuran tadpole/vitamin A/regeneration/homeotic transformation)

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In this study the effects of vitamin A on tadpoles of *Polypedates maculatus* with an amputated tail were investigated. After amputation of half the tail at the hindlimb-bud stage, tadpoles were exposed to vitamin A (palmitate) 10 IU/ml solution for 24 hr (Group I), 48 hr (II), 72 hr (III), 96 hr (IV), 120 hr (V) or 144 hr (VI). Vitamin A was deleterious to survival of the tadpoles since 10, 30 and 30% tadpoles died from Group IV, V and VI, respectively before the emergence of forelimbs. Regeneration of the tail was abnormal in 91.7% of the tadpoles. Vitamin A induced homeotic transformation of the amputated tail to hindlimbs in 11.6% of the animals. Normal limb development was suppressed partially or completely in 48.3% tadpoles. These results indicate that in addition to traumatic effects, vitamin A can lead to homeotic transformation of the tail into hindlimbs and can also interfere with normal development of limbs.

Introduction

Vitamin A and its derivatives, retinoids, have remarkable effects on different systems in the developing embryo. Local application of retinoic acid (RA) to the anterior side of a developing chick limb bud causes duplications in the anteroposterior (AP) axis of the limb (16, 17). In regenerating amphibian limbs, retinoids can lead to pattern duplication in the proximodistal (PD), AP and dorsoventral (DV) axes (2, 3, 4, 9). In contrast, treatment of regenerating tail with retinoids leads to inhibition of regeneration in *Bufo andersonii, Notophthalmus viridescens, Ambystoma mexicanum*, and *Xenopus laevis* (7, 12). However, in *Uperodon systoma* we found that in addition to inhibition of tail regeneration, limbs were generated at the site of tail amputation (6). This was the first clear demonstration of homeotic transformation in vertebrates. Our observations have recently been confirmed by Maden (5) who showed transformation of amputated tails into legs by retinyl palmitate in *Rana temporaria*. In this paper we report the homeotic transformation of tails into legs after vitamin A treatment in the Indian jumping frog *Polypedates maculatus*. In addition, we present a comparative account of the skeleton of the normal hindlimb and homeotically transformed hindlimb.

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Materials and Methods

Egg masses were collected from ponds, and tadpoles from the same egg masses were reared to the limb-bud stage in conditioned water and were fed with boiled *Amaranthus* greens and boiled egg *ad libitum*. Before amputation of half the tail, tadpoles were anesthetized with 1/3000 MS222. A control group of tadpoles was reared in conditioned water while experimental groups were exposed to 10 IU/ml vitamin A solution for 24 hours (Group I), 48 hr (II), 72 hr (III), 96 hr (IV), 120 hr (V) and 144 hr (VI), respectively. Vitamin A solution was prepared by dissolving vitamin A palmitate arovit (Roche) tablets in conditioned water. After exposure, the tadpoles were reared in conditioned water. Each group, consisting of 10 tadpoles, was kept in 500 ml of conditioned water, and were observed until the emergence of forelimbs. The snout to tail tip length of each tadpole was measured before amputation, after amputation and at the time of emergence of forelimbs. After emergence of forelimbs, the tadpoles were fixed in 10% formaldehyde solution, and their bone and cartilage were stained differentially by the alcian blue/alizarin red technique (18). Correlation coefficients of various parameters were calculated using standard statistical methods. Photographs were taken with a Pentax camera.

Results

The profiles of tail regeneration and limb development of individual tadpoles in control and experimental group are shown schematically in Figs. 1 and 3.

Controls

The mean snout to tail tip (ST) lengths before and after amputation were 24.5±1.8 and 14±0.6 mm, respectively. At the time of emergence of forelimbs, the mean ST length increased to 38.6±2.4 mm. All tadpoles showed normal tail regeneration (Fig. 2a), and there was no structural abnormality in the fore- and hindlimbs that emerged between day 21 and 30 (mean, day 27.4).

Tail regeneration in tadpoles treated with vitamin A for various periods

In Group I (24 hr treatment with vitamin A), 4 of 10 tadpoles showed normal tail regeneration (TP 6,

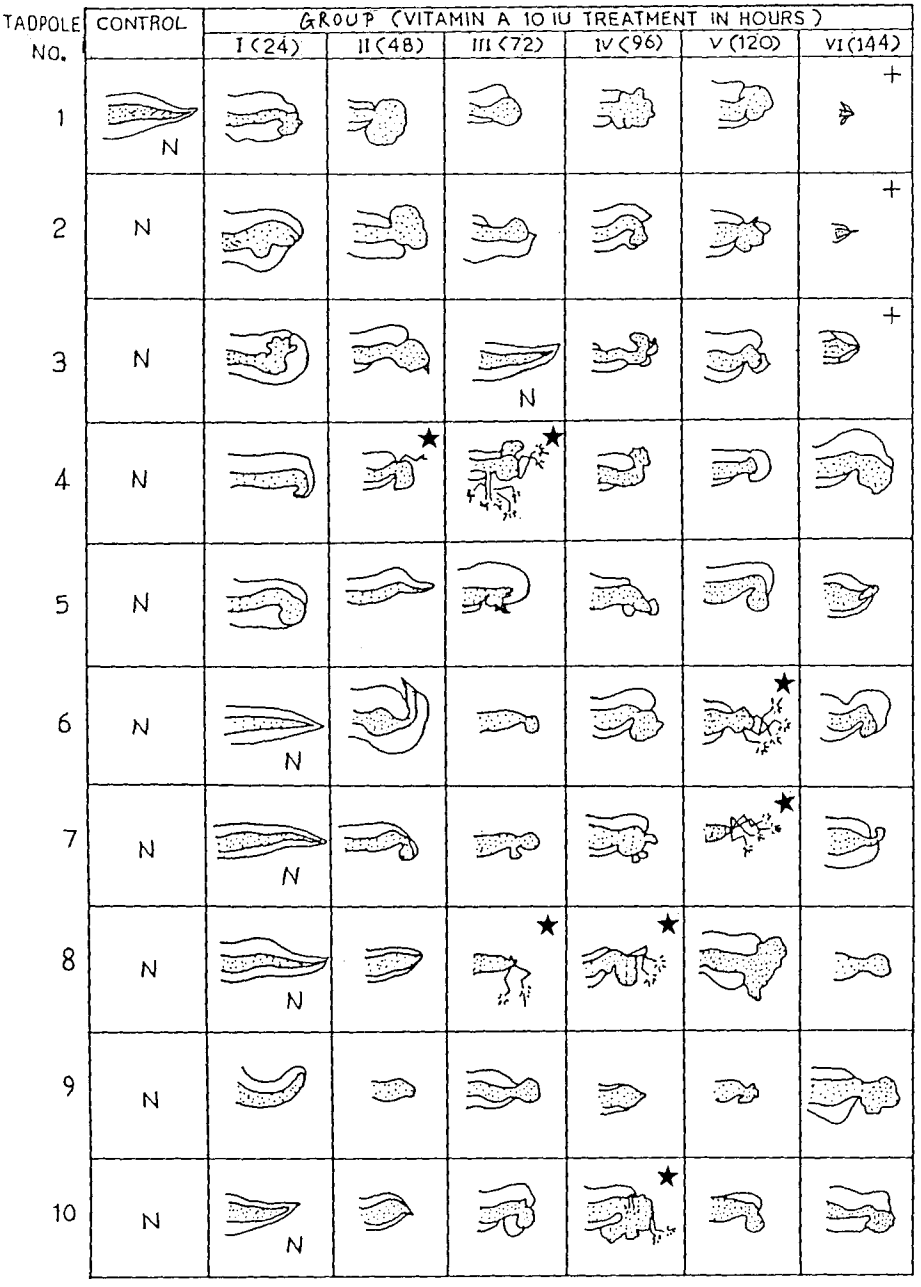

Fig. 1 Regenerated tails in individual tadpoles of *P. maculatus* following vitamin A (10 IU/ml) treatment for various periods (hrs) (see text for details). ★, tadpole with ectopic hindlimbs; +, died before limb development; N, normal tail regeneration.

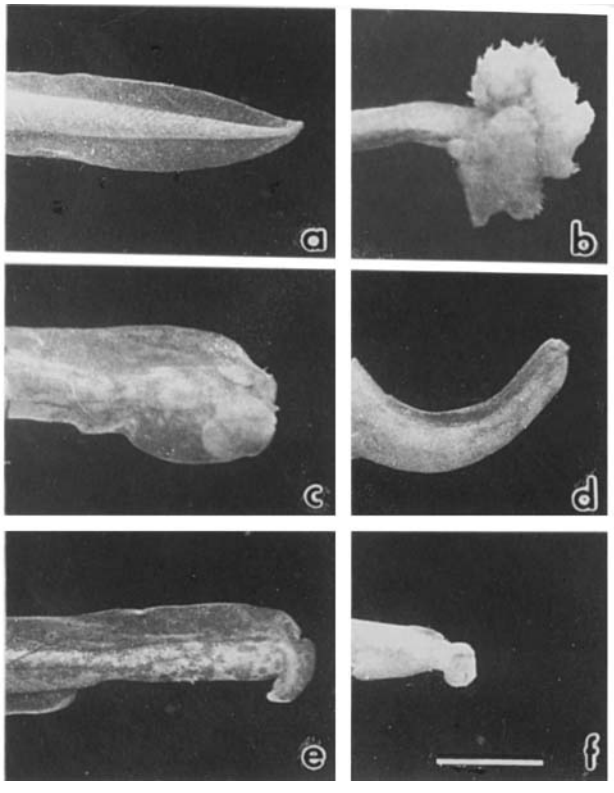

Fig. 2 Tail abnormalities in tadpoles after treatment with vitamin A (10 IU/ml) for various periods. a, Control, normal tail; b, 48 hr, bulbular mass at the tail tip; c, 24 hr, downwardly curved axial tissue; d, 24 hr, upwardly curved axial tissue; e, 48 hr, suppressed development of ventral tail fin; f, 144 hr, suppressed development of dorsal and ventral tail fins. Bar, 5 mm.

7, 8, and 10; Fig. 1). In other tadpoles the axial tissue of the regenerated tail was downwardly curved, becoming broader towards the tip (TP 1) or becoming broad at the distal end (TP 2). In these tadpoles, both dorsal and ventral tail fins developed, but did not cover the tail tip. Other abnormalities included upwardly curved axial tissue with the broad distal end (TP 3), downwardly curved axial tissue without ventral fin development (TP 4), and downwardly curved axial tissue with a bulbular distal end in which the fins failed to cover the tail tip (TP 5; Fig. 2c). In TP 9, the axial tissue was upwardly curved and only the dorsal tail fin developed (Fig. 2d). Interestingly, the dorsal tail fin developed in all the tadpoles to various extents, whereas development of the ventral tail fin was suppressed in 2 cases (TP 4 and 9).

In Group II (48 hr treatment with vitamin A), tail regeneration was abnormal in all 10 tadpoles (Fig. 1). A bulbular mass formed at the tail tip in 4 tadpoles (TP 1–4, Fig. 2b), whose dorsal and ventral tail fins developed, but did not cover the tail tip. Development of the ventral tail fin was suppressed in 2 tadpoles (TP 5 and 7; Fig. 2e), and development of tail fin was completely suppressed in one tadpole (TP 9). In TP 6, the axial tissue was laterally curved.

In Group III (72 hr treatment with vitamin A), one individual (TP 3) regenerated an apparently normal tail (Fig. 1). Bulbular growth of axial tissue

Table 1. Effects of vitamin A (10 IU/ml) on tail-amputated tadpoles of *P. maculatus*

Parameter	Group (time in hr of treatment with vitamin A)						
	Control (0)	I (24)	II (48)	III (72)	IV (96)	V (120)	VI (144)
Mortality before emergence of forelimbs (%)	0	0	0	0	10	30	30
Day[1] of death	—	—	—	—	28	30–49	4–6
Normal tail regeneration (%)	100	40	0	10	0	0	0
Ectopic hindlimb from tail (%)	0	0	10	20	20	20	0
Death before limb development (%)	0	0	0	0	0	0	30
Suppression of hindlimb development (%)	0	10	10	10	30	0	0
Suppression of forelimb development (%)	0	10	0	10	0	20	0
Partial suppression of limb development (%)	0	20	0	20	0	10	20
Total suppression of limb development (%)	0	10	40	40	20	0	10
Day[1] of emergence of forelimbs	21–30 (27.4)[2]	31–43 (35.6)	30–44 (39.8)	45–59 (50.4)	34–61 (45.4)	32–61 (46.4)	36–56 (46.3)
ST[3] length (mm) at time of emergence of forelimbs	38.6 (±2.4)[4]	34.8 (±4.5)	29.8 (±5.8)	28.7 (±4.5)	26.9 (±3.09)	25.7 (±4.1)	25.1 (±3.8)

1. Days after tail amputation.
2. Mean days from amputation to emergence of forelimbs.
3. Snout to tail tip length.
4. Standard deviation.

was observed in 6 tadpoles (TP 1, 4, 6, 7, 9, and 10), among which development of the tail fin was totally suppressed in 2 individuals (TP 6 and 7). Tail fin development was also completely suppressed in TP 8. In TP 1, 2, 4, 5, and 10, the tail fins on both sides developed partially but did not cover the tail tip.

In Groups IV, V, and VI (96, 120 and 144 hr treatment with vitamin A), 1, 3, and 3 tadpoles, respectively, died before emergence of forelimbs (Table 1). Tail regeneration was abnormal in all individual of these groups. In Group IV, both dorsal and ventral tail fins developed in all the tadpoles to various extents, but did not cover the tail tip (Fig. 1). A bulbular mass developed in 6 tadpoles (TP 1, 2, 6, 7, 8, and 10), among which downwardly curved axial tissue developed in 5 individuals (TP 2, 5, 6, 8, and 10). In 2 tadpoles (TP 3 and 4) axial tissue was curved dorsally.

In Group V, tail abnormalities included a

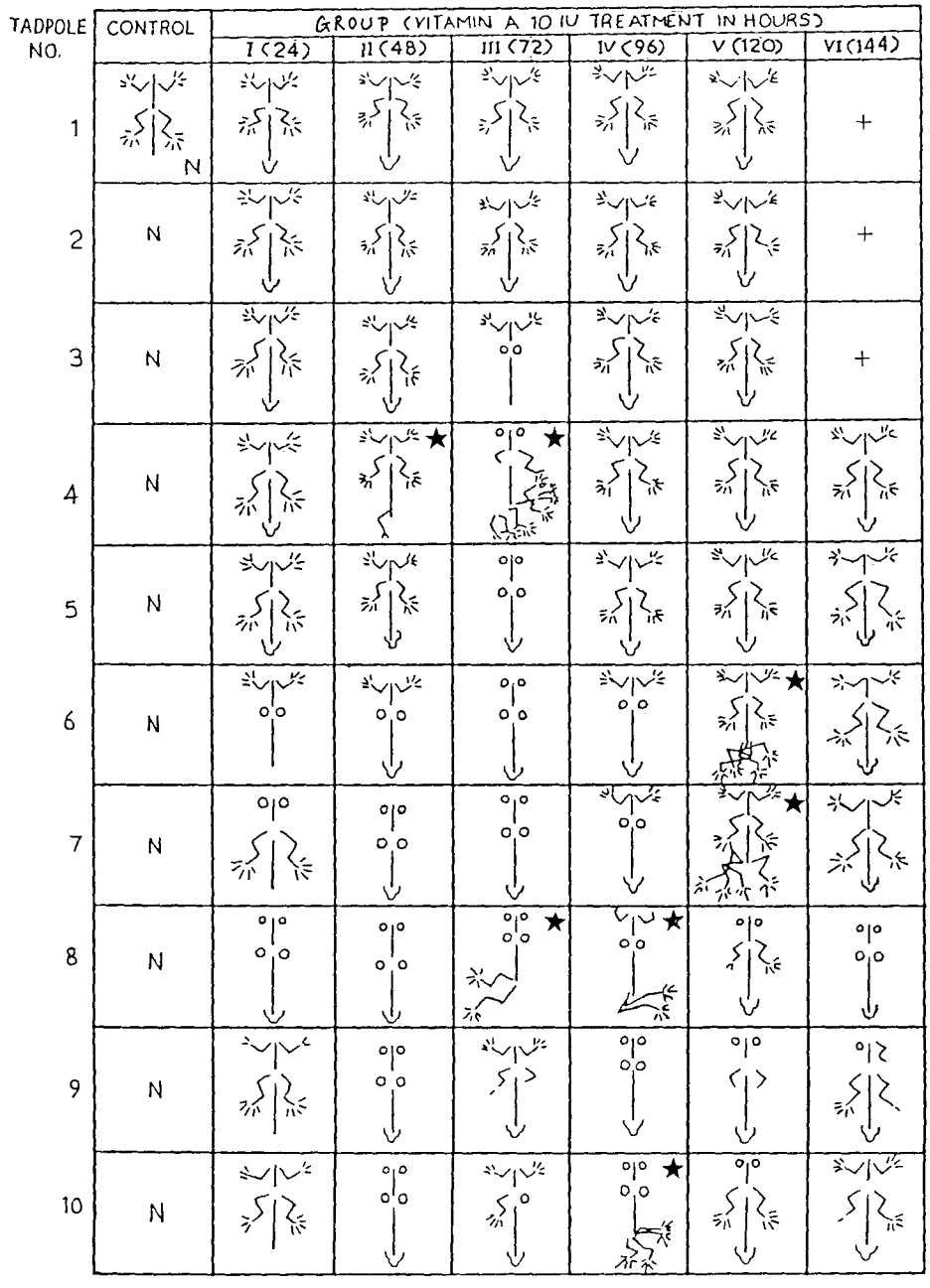

Fig. 3 Limb development in individuals following vitamin A (10 IU/ml) treatment for various periods (hrs) (ventral view). N, normal limbs; ○, suppressed limb development; ⟡, abnormal tail tip (see text for details); ★, tadpole with ectopic hindlimbs; +, died before limb development.

bulbular mass at the distal end of axial tissue in 5 individuals (TP 1, 2, 5, 6, and 10). In some animals, the axial tissue was upwardly (TP 1) or downwardly (TP 3, 5, and 10) curved. Suppression of tail fin development either partially (TP 5 and 10) or totally (TP 7 and 9) was also observed.

In Group VI, a pouch-like structure developed at the amputation site (TP 1 and 3), the distal end of axial tissue became bulbular in 3 tadpoles (TP 6, 9, and 10), including those in which it curved downward (TP 4, 6, and 10), upward (TP 7), or laterally (TP 5). Total suppression of tail fin development was seen in TP 8 (Fig. 2f).

Limb development in tadpoles treated with vitamin A for various periods

In Group I (vitamin A treatment for 24 hr), normal limb development occurred in 5 of 10 individuals (TP 1–5; Fig. 3). In other animals, suppression of development of hindlimbs (TP 6) or forelimbs (TP 7), or both (TP 8) was observed. Partial suppression of forelimb development also occurred, including development of two instead of four digits (TP 9) or three instead of four digits with right hindlimbs having three instead of five digits (TP 10).

In Group II (48 hr treatment with vitamin A), 5 individuals showed normal limb development (TP 1–5). Total suppression of limb development occurred in 4 individuals (TP 7–10), with suppression of hindlimb development in one (TP 6). In another animal (TP 4), tail regeneration was abnormal and an ectopic hindlimb resembling a normal hind limb with a thigh, shank, ankle, and two digits developed at the cut end of the tail.

In Group III (vitamin A treatment for 72 hr), normal limb development occurred in 2 individuals (TP 1 and 2; Fig. 3), total suppression of limb

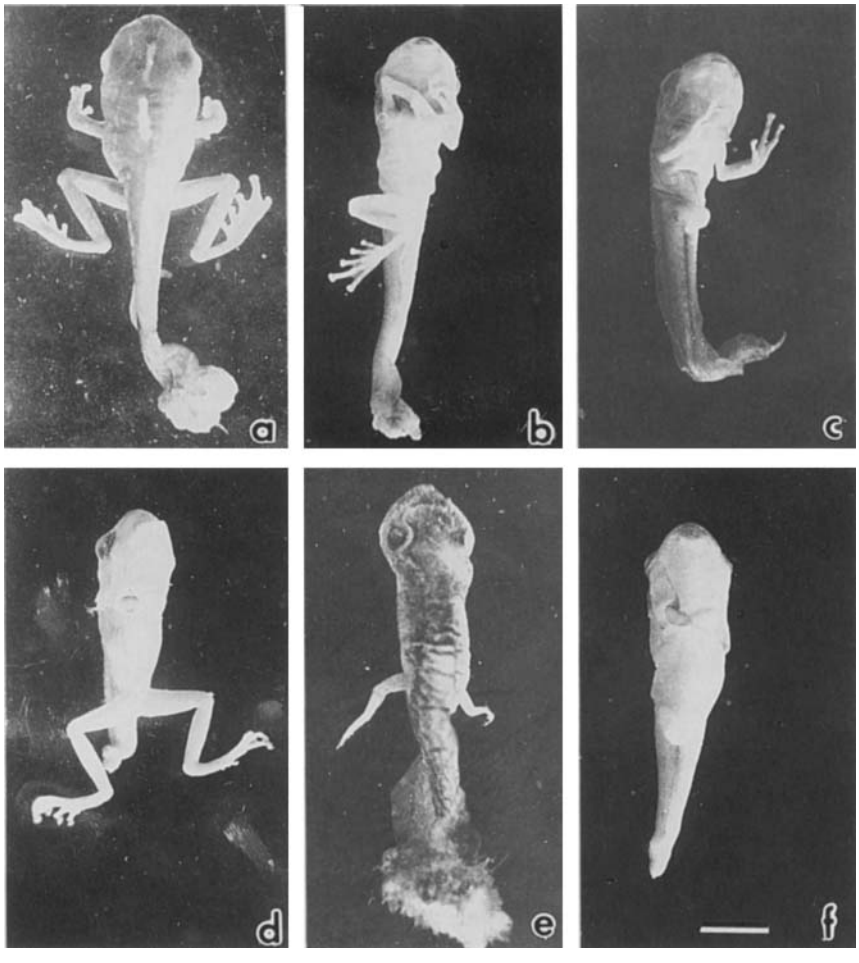

Fig. 4 Limb abnormalities in tadpoles with an amputated tail after vitamin A (10 IU/ml) treatment for various periods (hrs). a, 48 hr, normal limb development; b, 72 hr, suppressed development of left hindlimb; c, 120 hr, suppressed development of both hindlimbs; d, 120 hr, suppressed development of forelimbs; e, 120 hr, complete suppression of development of forelimbs and partial suppression of that of the right hindlimb; f, 144 hr, total suppression of development of fore- and hindlimbs. Bar, 5 mm.

development in 4 individuals (TP 5–8), and suppression of development of only hindlimbs in one individual (TP 3). The animals showing partial suppression included one (TP 9) whose left hindlimb developed up to the shank and the right hindlimb had one digit. In another animal (TP 10), the right forelimb had three digits instead of four and the left was complete, while development of the left hindlimb was totally suppressed but the right hindlimb was normal(Fig. 4b).

Ectopic hindlimbs developed from the amputated end of the tail in two individuals (TP 4 and 8) in Group III. In TP 4, a complete pelvic girdle and seven ectopic hindlimbs developed from the bulbular distal end of axial tissue (Figs. 5a, b, and 6). The first (from right to left) limb had three digits together with a distinct thigh and shank. Limbs nos. 2 to 7 had five digits each. In this tadpole development of both forelimbs was completely suppressed, and that of both hindlimbs was partially suppressed, the right one developing up to the shank and the left one having a single digit. In another animal (TP 8), two ectopic hindlimbs each having a thigh, shank, ankle and five digits

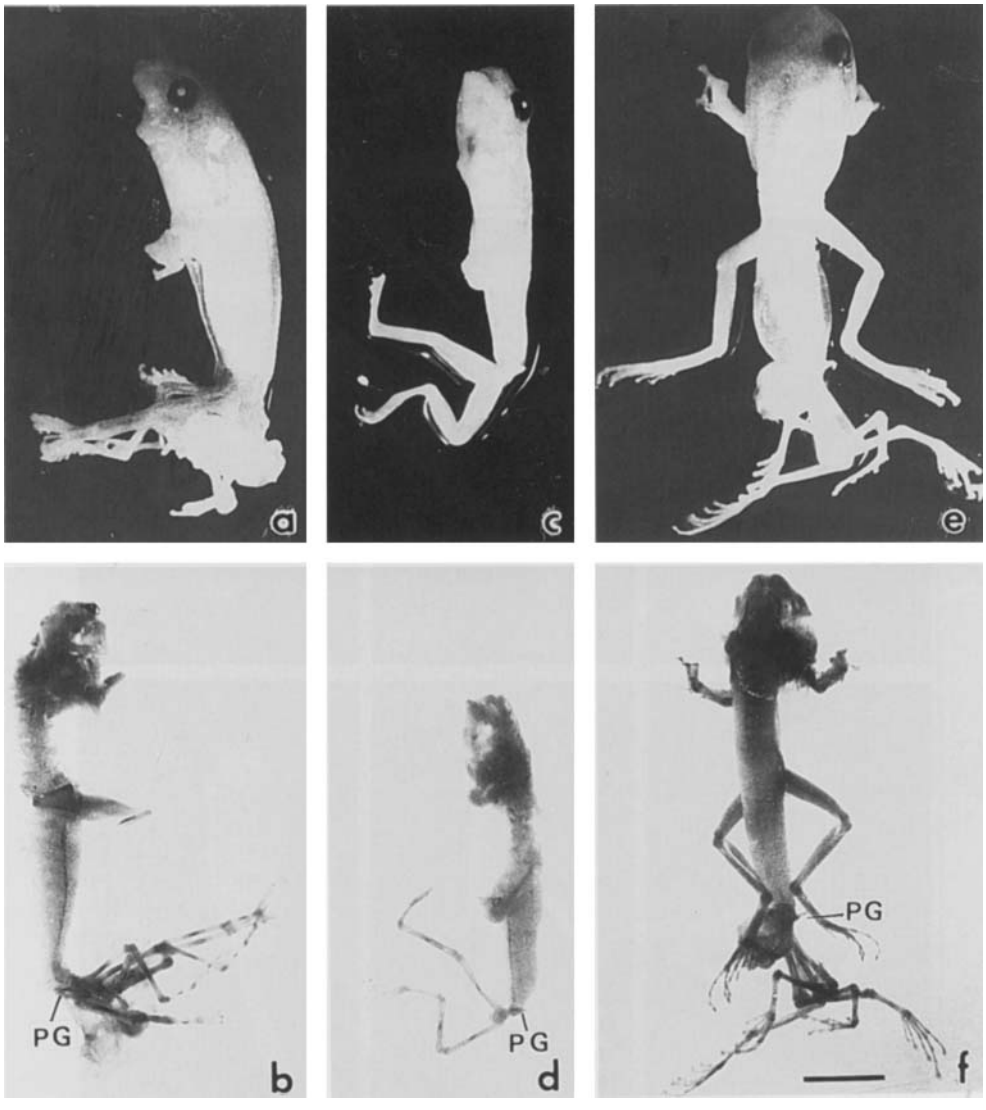

Fig. 5 Ectopic hindlimb (EHL) generation from amputated tail stumps in tadpoles following vitamin A (10 IU/ml) treatment for various periods (hrs). a, 72 hr, 7 EHL, forelimbs suppressed totally and hindlimbs partially; b, same tadpole showing skeletal development in EHL. The pelvic girdle (PG) is seen on both sides (note: only 6 limbs are visible, the smallest one being detached during processing); c, 72 hr, 2 EHL, suppression of fore- and hindlimb development (only stumps are seen); d, the same tadpole showing skeletal development in EHL (the PG has developed on one side); e, 120 hr, 4 EHL; f, the same tadpole showing skeletal development of EHL (the PG has developed on both sides). Bar, 5 mm.

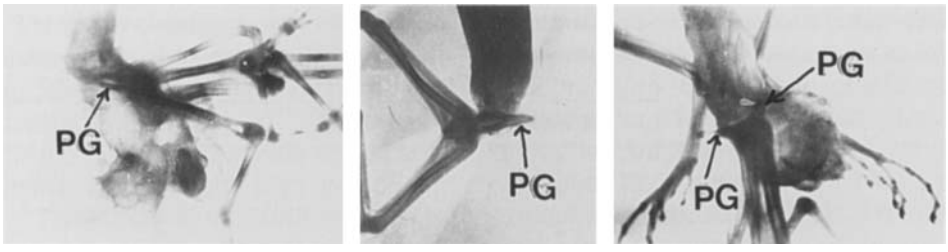

Fig. 6 Enlarged views of ectopic hindlimbs to show pelvic girdle (PG) formation (Corresponding tadpoles are shown in Figs. 5b, d, and f).

developed from the distal end of the tail, with a pelvic girdle on the right side only (Figs. 5c, d, and 6).

In Group IV (96 hr treatment with vitamin A), normal limbs developed in 5 out of 10 individuals (TP 1–5; Fig. 3). Hindlimb development was suppressed in 3 individuals (TP 6, 7, and 8), and limb development was completely suppressed in 2 individuals (TP 9 and 10). In TP 8, in addition to complete suppression of hindlimb development, both forelimbs had a single digit and thus their development was partially suppressed.

Ectopic limbs developed in 2 individuals (TP 8 and 10) in this group. In TP 8, two complete ectopic hindlimbs developed along with pelvic girdles on the right side. In TP 10, four ectopic hindlimbs developed, the first two with five digits each and the third and fourth had only four digits each.

In Group V (120 hr treatment with vitamin A), a normal orthotropic limb developed in 7 individuals (TP 1–7). The others showed abnormalities, including total suppression of forelimb development and suppression of hindlimb development to various extents. For instance, in TP 8, the right hindlimb had only two digits instead of five, whereas the left hindlimb developed five digits (Fig. 4e). The development of hindlimbs was normal in TP 10 (Fig. 4d), but in TP 9 both hindlimbs developed up to the shank only.

Prominent ectopic hindlimb development was induced in the cut end of the tail in 2 individuals (TP 6 and 7) in this group. TP 6 developed a complete pelvic girdle with four ectopic hindlimbs each having five digits (Fig. 5e, f, and 6). In TP 7 also, a complete pelvic girdle and four ectopic hindlimbs were formed, each having five digits, except limb 3 which had four digits.

In Group VI (144 hr treatment with vitamin A), 3 individuals (TP 1–3) died before limb development, and 4 (TP 4–7) developed normal limbs (Fig. 3). Total suppression of limb development was observed in TP 8 (Fig. 4f), and suppression of fore- and hindlimb development to various extents in the other 2. The right hindlimb had one digit instead of five in TP 10. In TP 9, development of the right forelimb was completely suppressed, the left forelimb had one digit instead of four, the right hindlimb was normal, but the left hindlimb had only one instead of five digits.

Analysis of ectopic hindlimbs (EHL)

There was a direct correlation between ectopic hindlimb formation and the duration of exposure up to 72 hr, the incidence being 10% on 48-hr exposure and increasing to 20% on 72-hr exposure (Table 1). On exposure for more than 72 hr, there was no change in the incidence of ectopic hindlimb formation. The correlation coefficient (r) between the time of exposure and EHL development was 0.385. There was a marked synchrony in the development of normal hindlimbs (NHL) and EHL in tadpoles that developed both types of limbs. The total length (proximal to distal) of EHL was less than that of the corresponding NHL of each individual. For example, the NHL length in Group II was 43.3, but that of EHL was 11.4 mm.

The patterns of bone and cartilage formations in the EHL were comparable with those in NHL. However, there were some differences in the skeleton of EHL from those of NHL. The skeleton of proximal parts (thigh and shank) was comparable but the number of skeletal elements were reduced in distal parts (digits) in EHL. Digit 1 and 2, which normally have three elements each, showed reduction to 2 (−1) or 1 (−2) (Table 2). For digit 3, the reduction ranged from −1 to −4, for digit 4 ranged from −1 to −5, and for digit 5 from −1 to −4. But, there was no direct correlation between the exposure time and degree of suppression of digital elements. However, for the anterior-most prehallux, there was a reverse relationship between the exposure time and degree of suppression, the prehallux being missing in 100, 100, 66.6 and 50% EHL in Groups I, II, III, IV and V, respectively. Thus, vitamin A interfered with the

Table 2. Comparison of skeleton of ectopic hindlimbs with normal hindlimbs in tail-amputated tadpoles of *P. maculatus*

Limb element	Normal hindlimb	Ectopic hindlimb (EHL) in Group			
		II (48)[1]	III (72)	IV (96)	V (120)
Thigh	1[2]	1	1	1	1
Shank	1	1	1	1	1
Ankle	2	2	2	2	2
Digit					
1	3	−2[3] (100)[4]	−1 (55.5) −2 (44.4)	−2 (66.6)	−1 (25)
2	3	−1 (100)	−1 (55.5) −2 (22.2)	−1 (50) −2 (16.6)	3[2]
3	4	−4 (100)	−1 (22.2) −2 (22.2)	−1 (16.6) −2 (50)	−1 (12.5)
4	5	−5 (100)	−1 (11.1) −2 (11.1) −5 (11.1)	−1 (16.6) −2 (16.6) −4 (33.3)	−2 (12.5)
5	4	−4 (100)	−1 (22.2) −4 (11.1)	−1 (16.6) −3 (16.6) −4 (33.3)	−4 (12.5)
Prehallux	1	−1 (100)	−1 (100)	−1 (66.6)	−1 (50)

Data were compiled for each group.
1. II-V, experimental groups (hours of treatment with 10 IU/ml of vitamin A).
2. Number of units in normal limb.
3. Reduction in number (−ve) from normal (*e.g.*, when the normal value is three units and EHL has only one, the value is expressed as −2).
4. Percentage of ectopic hindlimbs.

Table 3. Incidence of pelvic girdle (PG) formation with ectopic hind limbs (EHL) in tadpoles of *P. maculatus* with an amputated tail after vitamin A (10 IU/ml) treatment

EHL with	Group (time in hr of treatment with vitamin A)						
	Control (0)	I (24)	II (48)	III (72)	IV (96)	V (120)	VI (144)
PG on both sides	0[1]	0	0	10	0	20	0
PG on one side	0	0	0	10	10	0	0
No PG	0	0	10	0	10	0	0

1. Percentage of tadpoles.

development of distal as well as anterior elements of EHL.

Pelvic girdle formation was induced by 72 hr to 120 hr treatment and its extent of development seemed to be related to the duration of exposure as the incidence of its bilateral development was only 10% after 72-hr exposure and increased to 20% after 120-hr exposure (Table 3).

Discussion

Effects of vitamin A on development and metamorphosis

In this work we found a positive relationship between mortality and the duration of exposure to vitamin A, the correlation coefficient (r) being 0.872. The mortality was 10% after 96-hr exposure and increased to 30% after exposure for 120 or 144 hr (Table 1). Similar exposure-related mortality has been reported in *Bufo andersonii* (7, 11) and *Rana cyanophlyctis* (8). Interestingly, this correlation was not observed in *U. systoma* (6).

Metamorphosis was delayed by vitamin A treatment (Table 1). There was a positive correlation between the time of treatment and the onset of metamorphosis, correlation coefficient (r) being 0.789. The delay increased on exposure for up to 72 hr, but was constant on exposures for 72 hr to 144 hr. We have reported similar results for *U. systoma* (6). Niazi and Saxena (8) also reported prolongation of the life cycle in *R. cyanophlyctis* on exposure to vitamin A. They related this delay to decrease thyroid gland development, and reported that the thyroid gland became progressive-

ly smaller on exposure of tadpoles to higher concentrations of vitamin A. Histological examination revealed fewer follicle cells in the thyroid gland of the tadpoles treated with vitamin A. Possibly prolongation of the life cycle on treatment with vitamin A in the present study might also be due to suppression of thyroid gland development.

The ST (snout to tail tip) length at metamorphosis was also reduced by vitamin A treatment, the extent of reduction being directly related to the period of treatment (Table 1), the correlation coefficient (r) being −0.947. The ST length was greatest (38.6±2.4 mm) in tadpoles in the control group, and least (25.1±3.8 mm) in tadpoles in Group VI (144-hr treatment). These results are consistent with those on *U. systoma* (6), *B. andersonii* (7) and *R. cyanophlyctis* (8).

Tail regeneration was abnormal in 91.7% tadpoles in experimental groups. Up to 98.4% abnormality has been reported for *U. systoma* (6). Similar inhibition of tail regeneration after vitamin A treatment has been noted in *B. andersonii* (7, 10), *Xenopus laevis, Notophthalmus viridescens, and Ambystoma mexicanum* (12).

A pouch-like structure developed at the cut end of the tail early during regeneration in some experimental tadpoles. Folding of the fin to form a pouch-like structure at the tip of the regenerated tail has also been observed by Niazi and Saxena (7, 10) in *B. andersonii*.

The most unexpected finding was suppression of fore- and hindlimb development, ranging from partial to complete suppression (Fig. 3). The extent of suppression was maximal on 72-hr treatment (only 20% of the animals developed normal limbs) and was less on shorter (24 and 48 hr) or longer (96, 120 and 144 hr) exposure. Total suppression of development of both fore- and hindlimbs occurred in 40% of the animals exposed to vitamin A for 48 or 72 hr, but was less on shorter and longer exposures.

Dose- and exposure-time related suppressions of limb development by retinol palmitate have also been observed in larval *Xenopus laevis* and the axolotl *A. mexicanum* by Scadding and Maden (13, 14). They concluded that the suppression at higher concentrations was due to increase in the signal above the normal threshold level. The suppression of limb development in the present study probably also resulted from increase in the level of RA above the threshold level because of the additive effects of endogenous and exogenous RA. However, the recovery of the tadpoles on treatment for more than 72 hr is puzzling, and suggests that withdrawal of RA is more traumatic than continued exposure to it. On exposure to RA for more than 72 hr, there may be a drastic reduction in its endogenous level and so after treatment tadpoles may not be able to sustain limb development or recover from the trauma. However, if the treatment is continued for 96 hr, the tadpoles may take up within the threshold level of exogenous RA and so the percentages of total as well as partial suppression are decreased. On longer treatment, apparently recovery was even better as 70% of the animals developed normal limbs after 120 hr of exposure. Normal limb formation occurred in 40% of the animals on further exposure (144 hr), although 30% of the tadpoles in this group died at early stages.

According to Bryant and Gardiner (1), the application of RA to developing vertebrate limb buds causes all cells to be reprogrammed towards uniform positional values. Apparently, the lack of positional diversity in the progress zone leads to failure of growth and pattern formation and to the formation of reduced or truncated limbs. In the present study this reprogramming on vitamin A treatment may have led to suppression of limb development.

Induction of ectopic hindlimbs by vitamin A

Vitamin A induced ectopic hindlimb (EHL) development from the amputated tail stumps in *P. maculatus*. Similar induction of EHL has been observed in *U. systoma* (6), *R. temporaria* (5), *Bufo melanostictus* and *Microhyla ornata* (unpublished data). These results are difficult to explain, but evidently, in addition to the normal morphogenetic field for limb formation, other areas including the tail also have the capacity to form limbs. This indicates a broad morphogenetic field at the limb-bud stage, which probably becomes localized in the limb areas later on. The present results demonstrate a possible morphogenetic action of vitamin A on the blastemal cells of the tail as well as the cells of the limb-bud by some mechanism.

One class of molecules that are implicated in pattern formation, the capacity for regeneration and for carrying positional information are *Hox* genes. As mentioned by Maden (5), the *Hox* genes are likely to be components of homeotic transformation of the tail into legs. The genes that play important roles in establishment of axes and regeneration of limbs are the *Hox-4*, *Hox-3*, and *Hox-1* clusters (15). The *Hox-3* cluster is expressed specifically in normal and regenerating posterior appendages, like the hindlimbs and tail, and

is thought to provide positional memory for differentiation of the hindlimbs from the forelimbs. In homeotic transformation, tail is always converted into hindlimbs, so a gene of the *Hox-3* cluster may be involved in the development of the tail into legs. It is noteworthy in this connection that an effect of retinoids on *Hox-4.5* gene expression has been demonstrated by Simon and Tabin (15) in newt blastema. Therefore, vitamin A treatment may influence at *Hox* gene level, leading to suppression of limb development in the present study.

According to Bryant and Gardiner (1), the apparent homeotic change of the tail to limbs in regenerating frogs can be understood in terms of the effect of RA in changing pattern formation competent cells to a posterior-ventral-proximal (*i.e.*, flank) positional value, followed by interaction along the rostral-caudal axis of the body to generate two additional pairs of hindlimb sites on the tail. Although it is difficult to determine whether during homeotic transformation the tail tissue is directly converted to limbs or transformed into flank tissue first, as suggested by Bryant and Gardiner (1), there is circumstantial evidence in favour of the latter possibility. Invariably before generation of limbs, condensation of tissues takes place in a position anterior and lateral to the amputated tail tip. The EHL grows out of this mass in a ventrolateral direction, mostly in pairs, comparable and at times synchronously with the NHL. Sometimes a whole new tail regenerates dorsally and posteriorly to the EHL (unpublished results), showing that two distinct pathways are operative during the regeneration process. This is what is expected from the model proposed by Bryant and Gardiner (*loc. cit.*).

According to Maden (5) since homeotic transformation has been observed during the period of normal limb development and hindlimb development is induced by rising levels of thyroid hormone (TH), TH and specific thyroid hormone receptors (TRs) may be involved in homeotic transformation. Since, attempts to obtain homeotic transformations have failed in many other species (see 5 for details), Maden proposes that such transformation in *R. temporaria* and *U. systoma* may be due to higher levels of TH or even a novel TR. But, in *P. maculatus* the development of normal limbs, including the hindlimbs, was completely suppressed in tadpoles with homeotic transformation of the tail to limbs. This indicated that homeotic transformation can take place independently of limb development at normal sites. Therefore, the process of homeotic transformation may not be totally dependent on TH or TRs as proposed by Maden (5).

Thus, the present study in general confirmed the observations of others regarding the multiple and sometimes contradicting effects of vitamin A in different vertebrates, and demonstrated that homeotic transfer, first reported by us in *U. systoma* (6) and by Maden in *R. temporaria* (5), is also possible in *P. maculatus* in which amputated tails were converted to limbs mediated through vitamin A. As pointed out by Maden (5), on induction of homeotic transformation, large amounts of transformed tail tissue are generated and molecular analyses of this tissue may lead to the identification of the genes involved in vertebrates. Probing this tissue during this unique transformation may indicate which genes become silent, allowing transformation of tail tissue, and suppression of normal limb development, during the transition.

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