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Prevention of the effect of vitamin A palmitate on tail regeneration in toad tadpoles by sulphadiazine

(tail regeneration/Anura/vitamin A/sulphadiazine)

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ABSTRACT In *Bufo melanostictus* tadpoles treated with vitamin A palmitate after tail amputation, regeneration of notochord, spinal cord and muscles was inhibited, and the regenerating caudal fin formed an abnormal pouch. Sulphadiazine given together with vitamin A prevented the influence of the latter resulting in normal tail regeneration in many cases. This effect of sulphadiazine was subject to its amount relative to that of vitamin A used.

Vitamin A and its derivatives collectively called retinoids have profound influence on morphogenesis during development. Given in excess they cause a variety of malformations in mammalian foetuses[1], chick embryos[2] and also amphibian larvae[3-4]. Retinoic acid administered to chick embryos induces feather formation from scale producing leg epidermis[5] and duplication of digits in the wing[6]. In the amphibians retinoids produce interesting effects on tail and limb regeneration. Treatment of toad tadpoles with vitamin A palmitate after tail amputation inhibits regeneration of the axial tissues (notochord, spinal cord, muscles) but the caudal fin may regenerate in an abnormal manner forming a peculiar pouch on the right or left side of tail stump[7]. In the case of limb regeneration, retinoid treatment enhances the morphogenetic potential of the blastema radically changing the structural pattern of the resultant regenerate. Contrary to the rule, suitable retinoid treatment results in regeneration of a complete limb and also the girdle instead of only the part actually removed distal to amputation level[4, 8-11]. The mechanism by which retinoids produce specific effects on morphogenesis in developing systems is not yet known. However, there is sufficient evidence in favour of retinoids influencing gene activity[10,11]. Scadding[11] found that specific inhibitors of RNA and protein synthesis (actinomycin D, cyclohexamide and tunicamycin) antagonized the influence of vitamin A palmitate resulting in normal type of limb regeneration in axolotls. Recently, in this laboratory, Sharma and Archana[12] observed normal regeneration in many toad tadpoles immersed for 3 days after limb amputation in vitamin A palmitate suspension to which co-trimazine (sulphadiazine + trimethoprim) had also been added to prevent bacterial growth. This suggested probable antagonism between sulphonamides and vitamin A. In this communication we report that sulphadiazine given with vitamin A palmitate blocks the action of the latter on tail regeneration in toad tadpoles.

After amputation of distal 1/3 of the tail in 9 groups of *Bufo melanostictus* tadpoles group I (controls) was reared in plain tap water and groups II–IX were immersed for 3 days in tap water containing different amounts of either vitamin A palmitate

(VAP), sulphadiazine (SD) or both. Thereafter they were reared in plain tap water for the next 12 days. The chemicals employed were water dispersible vitamin A palmitate, type VII (Sigma) and finely powdered 400 mg tablets of sulphadiazine (May & Baker). The experiment was carried out at room temperature (28–30°C). Tadpoles were maximally fed freshly boiled spinach and media were changed daily.

In the control group I (Fig. 1) and also in groups II and III treated with SD alone, tail regeneration including that of axial tissues and the fin was completely normal in all cases. Treatment with VAP alone (groups IV, V) caused complete failure of tail regeneration (Fig. 2) or only the fin regenerated and formed the abnormal pouch (Fig. 3). In groups VI–IX treatment with VAP + SD produced 4 types of cases including those in which regeneration of both axial tissues and the fin (Figs. 4, 5) or at least of the fin (Fig. 6) was normal and others in which it was either completely inhibited or only the fin had regenerated abnormally forming a pouch. As the results presented in Table 1 show 0.1 and 0.5% amounts of SD given alongwith 1 IU/ml VAP resulted in completely normal tail regeneration in 17/20 and 18/20 cases of groups VI and VII, respectively, as opposed to absence of any such case in group IV treated with I IU/ml VAP alone. Results were not so spectacular when the same amounts of SD were given with 3 IU/ml VAP in groups VIII and IX. However, the antagonistic effect of SD was definitely apparent and its 0.5% amount was relatively more effective than 0.1% in preventing the influence of 3 IU/ml VAP on tail regeneration. Also, sulphadiazine prevented lethal effect of vitamin A on tadpoles. Whereas only 11/20 tadpoles of group V survived treatment with 3 IU/ml VAP alone, the number of survivors in groups VIII and IX given this amount of VAP together with 0.1 and 0.5% SD was 18/20 and 19/20, respectively.

The results show convincingly that SD added to the aqueous suspension of VAP in

Table 1–Effect of treatment of toad tadpoles with vitamin A palmitate and sulphadiazine separately and in combination on tail regeneration

Group	Treatment	Survivors	Types of regeneration among survivors (no.)			
			Normal		Only fin; abnormal pouch formed	Complete inhibition
			Axial tissues + fin	Only fin		
I	Nil	10/10	10	–	–	–
II	0.1% SD	20/20	20	–	–	–
III	0.5% SD	20/20	20	–	–	–
IV	1 IU/ml VAP	20/20	–	–	20	–
V	3 IU/ml VAP	11/20	–	–	5	6
VI	1 IU/ml VAP + 0.1% SD	20/20	17	–	3	–
VII	1 IU/ml VAP + 0.5% SD	20/20	18	2	–	–
VIII	3 IU/ml VAP + 0.1% SD	18/20	–	–	12	6
IX	3 IU/ml VAP + 0.5% SD	19/20	2	7	10	–

VAP = vitamin A palmitate; SD = sulphadiazine

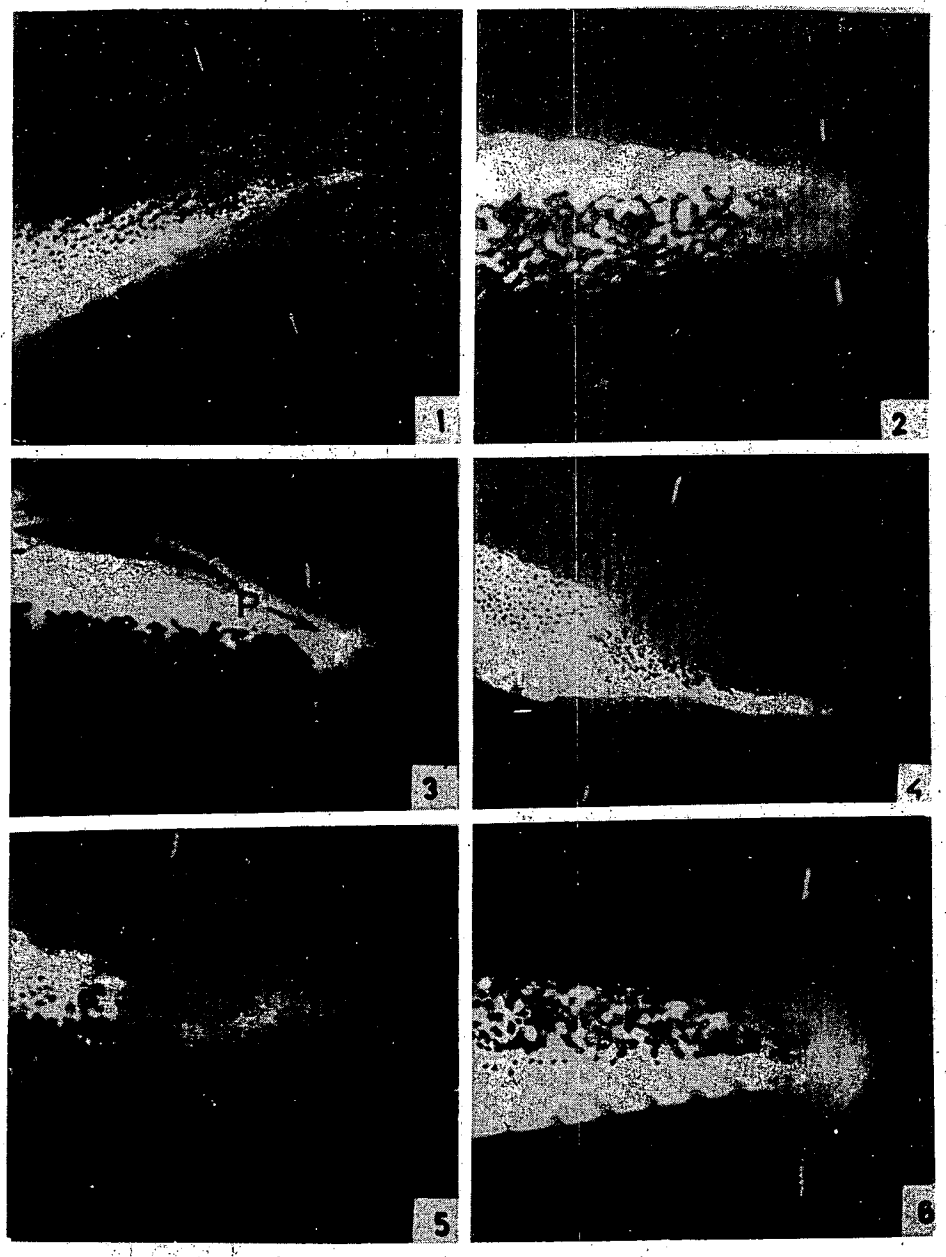

Fig. 1 A normal tail regenerate of control group I.
Fig. 2 Tail regeneration completely inhibited (Group V).
Fig. 3 Abnormal pouch (P) formed by regenerating fin (Group V).
Fig. 4 A normal tail regenerate of group VII.
Fig. 5 A normal tail regenerate of group IX.
Fig. 6 A case of group IX in which regeneration of fin was normal but that of axial tissues was inhibited. Lines indicate amputation level

which tadpoles are immersed, blocks the effects of the latter on tail regeneration and also the health of the tadpoles themselves. Effectiveness of SD appears to be subject to its amount relative to that of VAP in the medium. As mentioned earlier, similar preventive effect of co-trimazine was observed on limb regeneration in tadpoles of the same species. Antagonism between sulphonamides and vitamin A has never been reported before but our findings definitely indicate that these drugs interfere with utilization and/or metabolism of vitamin A palmitate in some way which needs to be investigated. Sulphonamides stop growth and reproduction of certain bacteria by preventing synthesis of folic acid and DNA[12]. This property certainly cannot be involved in prevention of vitamin A effect on regeneration which requires DNA synthesis and cell division. In any case animal cells utilize pre-formed folic acid and are independent of sulphonamide-sensitive synthesis. However, in mammals sulphadiazine is absorbed into blood from gastro-intestinal tract and binds with plasma proteins, especially albumin[13]. Vitamin A palmitate is also absorbed into blood by way of mucosal cells of the alimentary canal where it is first hydrolyzed into alcohol. It may be investigated whether sulphadiazine interferes with absorption of vitamin A palmitate through the cells of gastro-intestinal tract or its circulation in the blood.

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