Nerves innervate the ectopic limbs

Pragnya Das*,† and P. Mohanty-Hejmadi§

*Department of Zoology, Utkal University, Bhubaneswar 751 004, India
§Sambalpur University, Sambalpur 768 019, India

In this communication, we report the innervation of the ectopic hindlimbs formed as a result of homeotic transformation mediated by vitamin A in the Indian jumping frog, *Polypedates maculatus* (Anura: Rhacophoridae). Histology and nerve staining revealed that the ectopic limbs are innervated.

It is well known that vitamin A causes severe embryonic malformations in several animals ever since their discovery in 1909 (refs 1–3). However, its effect is more pronounced in amphibians causing proximodistal, anteroposterior and dorsoventral duplication of parts of the limbs during limb regeneration in several species of amphibians[4–14]. But the most remarkable of all the effects of vitamin A is the homeotic transformation of tails to limbs in *Uperodon systoma*[15]. Since then several workers have confirmed similar phenomenon in other species of amphibians[16–19]. As many as 8–9 ectopic limbs arise from the tail tissue. The limbs arise either singly or in pairs. Most of the ectopic limbs have the normal hindlimb structure but none of them is functional. The tadpoles dragged the extra limbs and died as a result of overgrowth. As the ectopic limbs were functionless, it was possible that they were devoid of nerves. The present study was therefore undertaken to ascertain whether the ectopic limbs of *P. maculatus* were supplied with nerves.

The egg mass of *P. maculatus* was collected from Utkal University campus in July, 1995 and reared in the laboratory up to the hindlimb bud stage following the standardized procedure of Mohanty-Hejmadi[20]. Prior to amputation in the middle of the tail, the tadpoles were anaesthetized in MS 222 (tricaine methanesulphonate) and exposed to vitamin A 10 IU/ml for 72 h. Following the above treatment they were transferred to aerated, conditioned water and allowed to grow till the emergence of ectopic limbs. Throughout the experiment they were fed with boiled egg and *Amaranthus ad libitum*. Once the ectopic limbs had developed and the forelimbs emerged, the tadpoles were fixed in 10% buffered formalin.

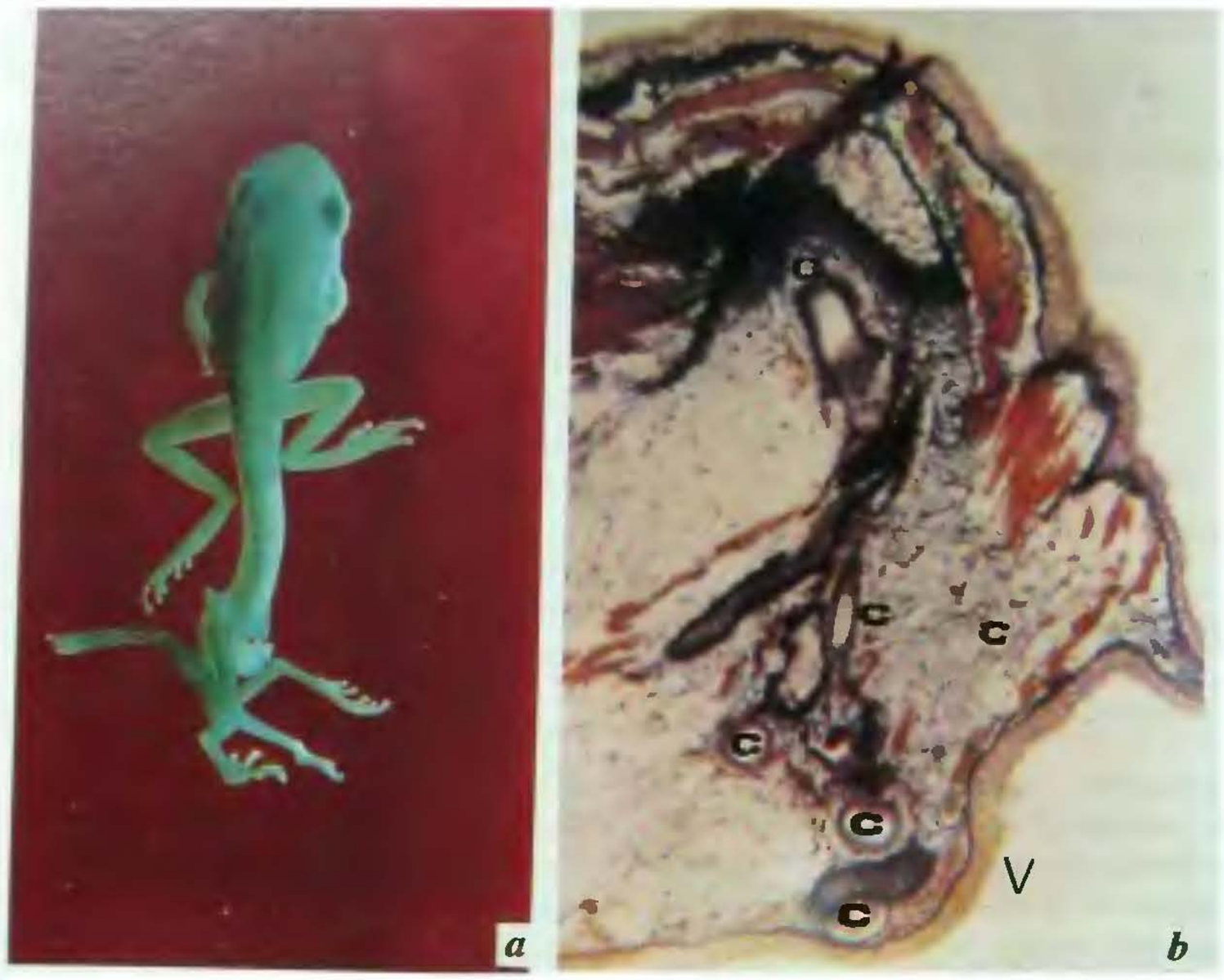
For histological studies of the tail region of tadpoles with ectopic limbs, the tails of those tadpoles who possessed ectopic limbs (Figure 1 *a*) were amputated and fixed in aqueous Bouin's fluid, embedded in paraffin (m.p. 58°C–60°C), sectioned longitudinally at 10 µm thickness and stained with Mallory's triple stain for examination under the light microscope.

Interestingly, histological studies revealed that multiple sections of the nerve cord were found in the extreme distal and ventral regions of the tail (Figure 1 *b*). As the limbs always arise from the ventral side, it is quite likely that the nerve cord which is located on the dorsal side, takes several turns in the distal region of the tail to reach the ventral side, perhaps to supply the ectopic limbs. As a result, multiple sections of the nerve cord were visible on the ventral side in the distal region of the tail.

To be more sure that the ectopic limbs were innervated, Sihler's differential nerve staining technique[21] was used on the tadpoles with ectopic limbs. In both the tadpoles (Figure 1 *c*, *d*) a thin, faint nerve could be discerned in the ectopic limbs. The continuity of the nerves could not be tracked due to the shrinking of the specimens during fixation. On the other hand, the nerves innervating the normal hindlimbs were thick and prominent (Figure 1 *c*) and were therefore easily distinguishable.

The present study therefore confirms that, because of sparse and weak innervation the ectopic limbs are not fully functional, although morphologically they are well-developed hindlimbs. They are richly vascularized too as revealed from histology (unpublished data). Singer[22] transplanted the upper arm segments of the adult *Notophthalmus viridescens*, in the flank region of the same animal and found that a few of the grafts regenerated after some delay. The arms which had regenerated contained an average less than one-third of the normal density of nerve fibres. A similar fraction of the total nerve supply can induce the formation of supernumerary limbs when diverted to a surface wound. Thornton and Tassava[23] also recorded regeneration in orthotypically transplanted arms of *Ambystoma mexicanum* larvae which were kept sparsely innervated by repeated denervation. On the other hand, if the hind brain and trunk nerve cord are excised when they first form a neural tube, a defective embryo develops which survives and develops normally but is incapable of normal movements or feeding. The arms develop quite well with either very few or no detectable nerve fibres and can reach the four digit stage in this species[22]. This might be the

†For correspondence.



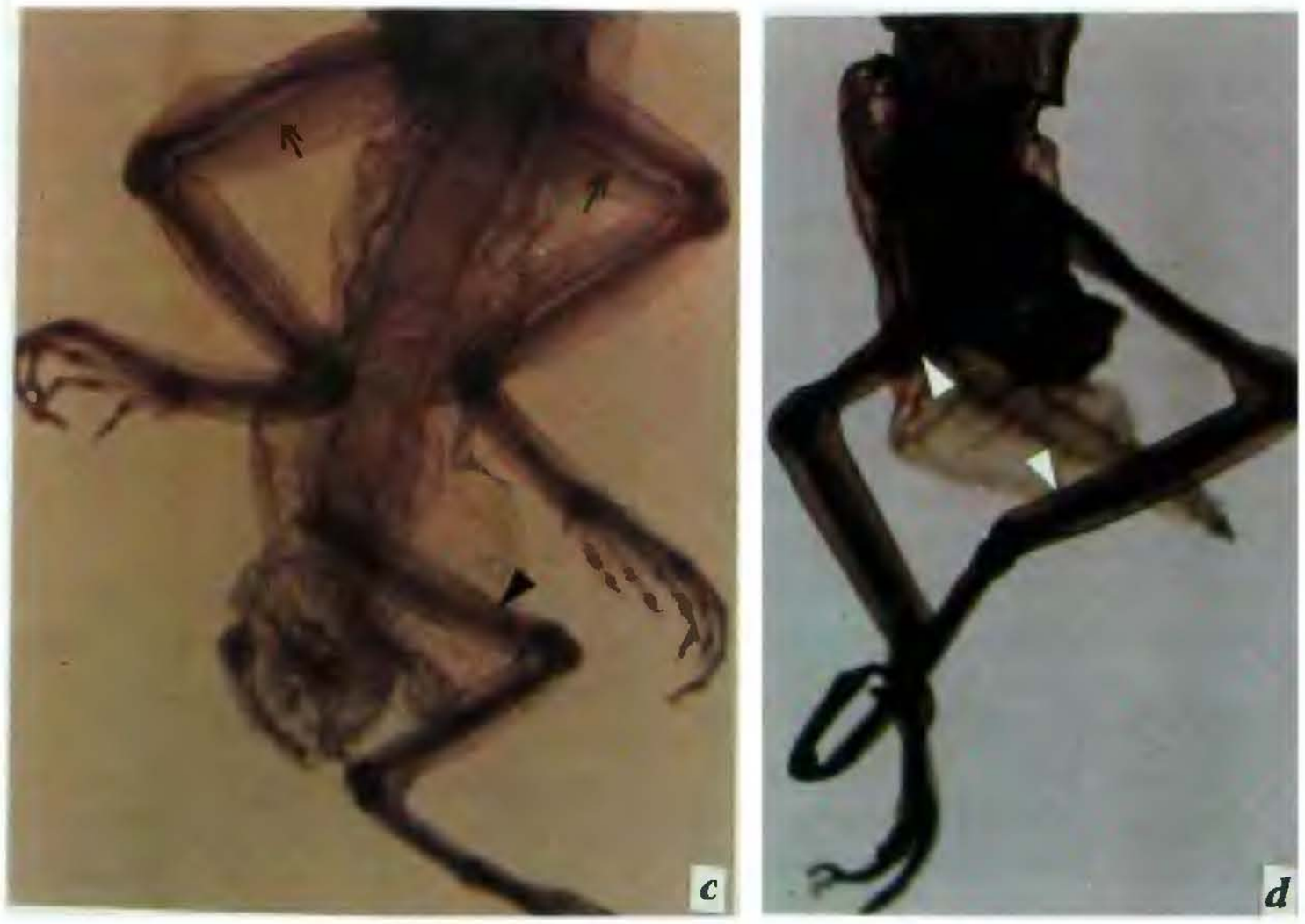


Figure 1. *a*, Supernumerary ectopic limbs in the tail amputated tadpoles of *P. maculatus* following exposure to vitamin A 10 IU/ml for 72 h. The ectopic limbs were fully developed after 35–40 days of the initial amputation (× 10). *b*, Longitudinal section of vitamin A treated tail showing multiple sections of the nerve cord (C) on the ventral side (V) of the distal region of the tail. The nerve cord, supposed to be on the dorsal side is seen here taking a turn from the dorsal side to the ventral side (× 100). *c*, Sihler's differential nerve preparation shows a thin nerve (marked by arrowhead) in the single ectopic limb of *P. maculatus*. The normal hind limb shows a thick and distinct nerve (marked by arrow) (× 11). *d*, Sihler's differential nerve preparation shows a thin nerve (marked by arrowhead) in the paired ectopic limbs of *P. maculatus* (× 11).

reason why the ectopic limbs formed in *P. maculatus* in the present study though seemed normal, may not be fully functional because they are sparsely and scantily innervated.

1. Pawson, B. A., *Ann. N. Y. Acad. Sci.*, 1981, 359, 1–8.
2. Roberts, A. B. and Sporn, M. B., in *The Retinoids* (eds Spom, M. B., Roberts, A. B., and Goodman, D. S.), Academic Press, Toronto, 1984, vol. 2, pp. 209–286.

3. Sporn, M. B. and Roberts, A. B., in *Retinoids, Differentiation and Disease*, IBA Found. Symp., 1985, vol. 113, pp. 1–5.
4. Niazi, I. A. and Saxena, S., *Folia Biol.*, 1978, **26**, 3–11.
5. Maden, M., *Nature*, 1982, **295**, 672–675.
6. Maden, M., *J. Embryol. Exp. Morphol.*, 1983, **77**, 273–295.
7. Maden, M., *Dev. Biol.*, 1983, **98**, 409–416.
8. Thoms, S. D. and Stocum, D. L., *Dev. Biol.*, 1984, **103**, 319–328.
9. Kim, W. S. and Stocum, D. L., *Wilhelm Roux's Arch. Dev. Biol.*, 1986, **195**, 455–463.
10. Kim, W. S. and Stocum, D. L., *Dev. Biol.*, 1986, **114**, 170–179.
11. Scadding, S. R. and Maden, M., *J. Embryol. Exp. Morphol.*, 1986, **91**, 19–34.
12. Scadding, S. R. and Maden, M., *J. Embryol. Exp. Morphol.*, 1986, **91**, 35–53.
13. Scadding, S. R. and Maden, M., *J. Embryol. Exp. Morphol.*, 1986, **91**, 55–63.
14. Koussoulakos, S., Kiortsis, V. and Anton, H. J., *IRCS Med. Sci.*, 1986, **14**, 1093–1094.
15. Mohanty-Hejmadi, P., Dutta, S. K. and Mahapatra, P., *Nature*, **355**, 352–353.
16. Maden, M., *Dev. Biol.*, 1993, **159**, 379–391.
17. Muller, G. B., Streicher, J. and Wurm, R., Proc. 2nd World Congress, Australia, 1993, pp. 176–177.
18. Mahapatra, P. and Mohanty-Hejmadi, P., *Dev. Growth. Differ.*, 1994, **36**, 307–317.
19. Das, K. and Dutta, S. K., *Curr. Sci.*, 1996, **71**, 61–65.
20. Mohanty-Hejmadi, P., *Prakruti-Utkal Univ. J. Sci.*, 1977, **11**, 81–87.
21. Williams, T. W., *Anat. Rec.*, 1943, vol. 86, no. 2.
22. Singer, M., *Am. Zool.*, 1978, **18**, 829–841.
23. Thornton, C. S. and Tassava, R. A., *J. Morphol.*, 1969, **127**, 225–232.

ACKNOWLEDGEMENTS. The help extended by Mr D. P. Rath, Wildlife Institute of India, in the preparation of the photographs is gratefully acknowledged.

Received 17 December 1997; accepted 29 January 1998

MEETINGS/SYMPOSIA/SEMINARS

International Symposium on Apoptosis

Date: 30–31 October 1998
Place: Chandigarh, India

The symposium will provide a forum for the cell and molecular biologists working in the area of 'Programmed cell death'. The symposium, besides deliberating on the basic advancement in our understanding of the subject, will also focus on the 'Apoptosis-targeted therapies' as the most promising goal of this chosen frontier of Biomedical Science.

Contact: Dr S. N. Sanyal
Organizing Secretary
International Symposium on Apoptosis
Department of Biophysics
Panjab University
Chandigarh 160 014, India
Tel: 91-172-541441 extn 1364
Fax: 91-172-545459
E-mail: Pulib@puniv.chd.nic.in

35th Annual Convention and Meeting on Continental Margins of India – Evolution, Processes and Potentials

Date: 18–20 November 1998
Place: Dona Paula, Goa

Besides the special theme 'Continental Margins of India – Evolution, Processes and Potentials', original research contributions are invited for presentation in the following fields: Imaging techniques in exploration; Integrated geophysical techniques; Solid earth geophysics; Geophysical exploration; Marine geosciences; Atmospheric sciences; Geodynamics; Space sciences and planetology.

Contact: Dr P. R. Reddy
Honorary Secretary
Indian Geophysical Union
NGRI Campus, Uppal Road
Hyderabad 500 007
Phone: (040) 7172911
Fax: (040) 7171564
E-mail: postmast@csngri.ren.nic.in

Pharmaceutical Education. Dr Harkishan Singh. Vallabh Prakashan, SU-221, Pitampura, Delhi 110 034. Price: Rs 350, $ 40. 204 pp.

Pharmacists are recognized world over as pivotal players in the healthcare system, primarily responsible for the final quality and therapeutic efficacy of administered medicaments, and, consequently, the education they receive must equip them to contribute at one or several stages of the 'laboratory-to-clinic' journey of a pharmaceutical product. These stages include: (a) the new drug discovery process; (b) the design, development and manufacture of pharmaceutical dosage forms; (c) optimization of therapy in the hospital/clinic; (d) dissemination of product information to the practising physician and community at large, and (e) the drug regulatory process which ensures the final quality, safety and efficacy of the pharmaceutical product.

Education in the pharmaceutical sciences and technology is therefore multidisciplinary and necessitates inputs from both basic and applied sciences relevant to the drug discovery process and the subsequent development, evaluation and clinical performance of a new drug molecule and/or its pharmaceutical products. The education system must therefore provide not only multiple avenues for specialization but also a comprehensive foundation. This necessitates the development of a system which is liberal enough to incorporate and assimilate all the relevant streams of science and technology. With the increasingly proliferating knowledge base and the concomitant fading of barriers between scientific disciplines, education must know no barriers and the education system must be flexible and open to a continuous interdisciplinary exchange of knowledge, expertise and people. This is of particular importance for interdisciplinary sciences such as the pharmaceutical sciences.

Harkishan Singh informs us that pharmaceutical education in India is imparted through a vast network of 290 diploma institutions, 62 degree institutions and approximately 30 postgraduate programmes, with the objective of either qualifying registered pharmacists (diploma) or providing trained manpower to the pharmaceutical industry (degree). The role of hospital pharmacy and clinical pharmacy, both in the curriculum as well as in practice, however, still remains to be defined.

While the diploma and degree institutions, both government and private, have flourished in India, the evolution and progress of postgraduate education has been painfully slow, hampered by lack of direction and national will. Singh narrates the events that finally led to the establishment of India's first National Institute for Pharmaceutical Education and Research, NIPER, at Mohali in Punjab, as late as 1993–94, even though the idea was mooted in the 1950s, when the All India Institute of Medical Sciences and the five Indian Institutes of Technology were set up.

Till date, despite the increasing demand for pharmacy graduates and pharmaceutical scientists, there appear to be no plans by the central government to set up other such institutes in the country. The state governments are strangely reticent on the subject as their portfolios and budgets permit a rather short-sighted view of postgraduate education, de-linked from research. It is ironic that only in case of the pharmaceutical sciences have the policy makers and educationists of this nation failed to recognize the relevance of a sound scientific research base as a pre-requisite for quality education, and this is despite a flourishing pharmaceutical industry in India and the increasing importance of pharmaceutical R&D both in India and abroad, in the era of globalization, with India a signatory to GATT and WTO.

The setting up of the B.V. Patel PERD Centre in 1991, in Ahmedabad, represents the first initiative of its kind by the pharmaceutical industry and a state government to establish a postgraduate research institute of excellence. However, without the continued financial support of the industry and recognition and support by the Centre, this maiden venture maybe starved and its growth and development hampered. Postgraduate research and education in the pharmaceutical sciences is clearly at a crossroad and awaits its rightful place on the national agenda.

Much of this and more is the subject of Singh's treatise on pharmaceutical education, as he traces the development of diploma, degree and postgraduate education and research since the introduction of pharmacy education in 1860 at the Medical College, Madras, culminating with the establishment of NIPER and B.V. Patel PERD Centre in the early 1990s. The format of the book provides details of important landmarks and a chronological listing of events that led to the establishment of the major pharmacy degree and postgraduate institutes, with due recognition of the individuals who have contributed to the development of these educational institutes as well as played a role in delineating the policies that have influenced the course of pharmaceutical education. Singh has also highlighted the role of professional and statutory policy-making bodies, the Pharmacy Council of India (PCI), the All India Council of Technical Education (AICTE) and the various advisory committees which were set up by the Central Government, post-independence, to frame the health policies of the nation as well as the agenda for imparting technical education, to train manpower for industrial growth and development.

The most significant outcome of Singh's book is the issues that have emerged unresolved, which demand a solution from the educationists, the profession and national policy makers. These include: (a) the need to delineate two distinct paths of pharmaceutical education and training – for registered pharmacists and hospital and clinical pharmacists, and for the pharmaceutical industry and pharmaceutical research; (b) the debate on whether diploma education is adequate or be replaced by a 4-year-degree course as a minimum qualification for registered pharmacists; (c) the ambiguous and inadequately defined roles of PCI and AICTE, the two bodies which control the quality of education imparted; (d) the necessity of carving a niche for the pharmaceutical sciences by establishing separate pharmacy faculties, instead of subjecting pharmaceutical education to a tug-of-war between the Education Commission in Health Sciences and AICTE, neither of which can adequately cater to the needs of pharmaceutical education with its multidisciplinary approach; (e) the importance of developing at least one model institution in each State, for providing courses of study in pharmacy, along the lines of IITs and IIMs; (f) strengthening of doctoral and postdoctoral research programmes; and (g) positioning pharmacy on the national health, science and technology agenda, in recognition of its importance in the national and international scenario.