

these plants and gradually improved upon them over several millennia. That is a wonderful concept, but is unworkable for all generic inventions if taken to its logical conclusion – because logically then, every intermediary in the improvement process must also be entitled to a share of the IPR. I illustrate its unworkability with an example from medicine.

It was known several centuries ago to the Anglo-Saxon community that leaves of the foxglove plant are useful in the treatment of dropsy (heart failure). From this knowledge came the discovery of the digitalis alkaloid and then of digoxin, followed by the identification of the digoxin receptor and then new-generation synthetic drugs that act on the receptor. Swaminathan's prescription will mean that a fraction of the IPR on the latest drugs will return to the Anglo-Saxon communities, but what then of the other intermediaries in the evolution of the invention?

It would appear, therefore, that the concept of limited-term monopoly followed by transfer of the knowledge to the public domain is an equally reasonable and a more workable solution. Consequently, an important corollary to my position is that India may adopt the patent protection mode even for conferring IPR on new plant varieties instead of considering alternative *sui generis* systems.

Finally, how would an IPR regime as the one argued for above affect the economy of India? I confess that I am not an expert to answer this question, and it is quite possible that our country should not adopt such a regime because it will be economically harmful for our countrymen. In that case, however, the economic justification for not adopting IPR for life forms should clearly be spelt out and the reasons why it will be disadvantageous for the country be cogently argued. Such a decision will then reflect economic realities, which will be used to con-

sciously override the scientific arguments presented here.

As indicated above, it may not be sufficient to make the case that patents in general are economically harmful and therefore that patents on life forms should be disallowed. One also runs the risk of being accused by other nations of being insensitive to the issues of promoting multilateral trade and hence of being subjected to sanctions, which may prove to be more economically ruinous in the long run. Thus, to exclude life forms from IPR on 'scientific grounds' will be an instance of using a false proxy to defend oneself in what is really a socio-economic disagreement between the world's trading nations.

---

*J. Gowrishankar is in the Centre for Cellular and Molecular Biology, Hyderabad 500 007, India.*

---

## SCIENTIFIC CORRESPONDENCE

### Polyamine biosynthetic pathway: A potential target for plant chemotherapy

The discovery of polyamines stemmed from the observations of crystals of a polyamine from human semen in 1678 by Antoni van Leeuwenhoek. Later in 1888, these crystals were identified as an organic base and given the name spermine<sup>1</sup>. After a long gap, the reemphasis on polyamine perspectives in biology began in the 1960s and 1970s, with the accumulation of data on their role in cell proliferation and differentiation<sup>1,2</sup>. However, this area of research has grown in significance in the last 10 years (especially from molecular biology) as these naturally occurring polycationic small ubiquitous molecules play a pivotal role in diverse cellular and molecular processes such as the regulation of cell division, growth and development, membrane stability, synthesis and function of DNA, RNA and proteins in many organisms, including plants<sup>3</sup>. It has been suggested that polyamines could be treated as a new class of 'intracellular growth regulators' or second messengers<sup>4</sup>. Although the

mechanism of action of polyamines in various cell functions is not clearly known, (especially in plants), the polyamine biosynthetic pathway is fairly well established<sup>3</sup>.

The most common polyamines are putrescine (diamine), spermidine (triamine) and spermine (tetraamine). Putrescine can be formed by two biosynthetic pathways, either directly from decarboxylation of L-ornithine by ornithine decarboxylase (ODC) or indirectly from L-arginine decarboxylation by arginine decarboxylase (ADC) through a couple of intermediates. Spermidine and spermine are synthesized by the addition of an aminopropyl group [donated by decarboxylated S-adenosylmethionine (SAM) formed from decarboxylation of SAM by SAM decarboxylase] to one or both primary amine group of putrescine by spermidine and spermine synthases, respectively<sup>1</sup>. The specific inhibitors are available for the enzymes involved in polyamine biosynthesis<sup>5</sup> (Figure 1). For

instance, difluoromethylornithine (DFMO) and difluoromethylarginine (DFMA) specifically and irreversibly inhibits ODC and ADC, respectively<sup>5</sup>. Both these pathways operate in plants and bacteria<sup>1</sup>, but pathogenic fungi<sup>6-8</sup>, with a few exceptions<sup>9,10</sup> and most probably protozoa<sup>11</sup> and insects<sup>12</sup> possess only an ODC pathway for polyamine biogenesis as in case of animals and humans<sup>13</sup>. Since a majority of fungi are dependent on ODC pathway for polyamine formation, which is an absolute requirement for normal fungal growth and development, the specific inhibition of fungal polyamine biosynthesis using ODC inhibitors like DFMO should be lethal<sup>14</sup>. In fact, this was the basis for the discovery of control of a plant disease by selective inhibition of fungal polyamine biosynthesis, without affecting polyamine biosynthesis, growth and development of the host plant as it contains an alternative ADC pathway for polyamine formation<sup>15</sup>. Previously, selective targeting of polyamine biosynthetic

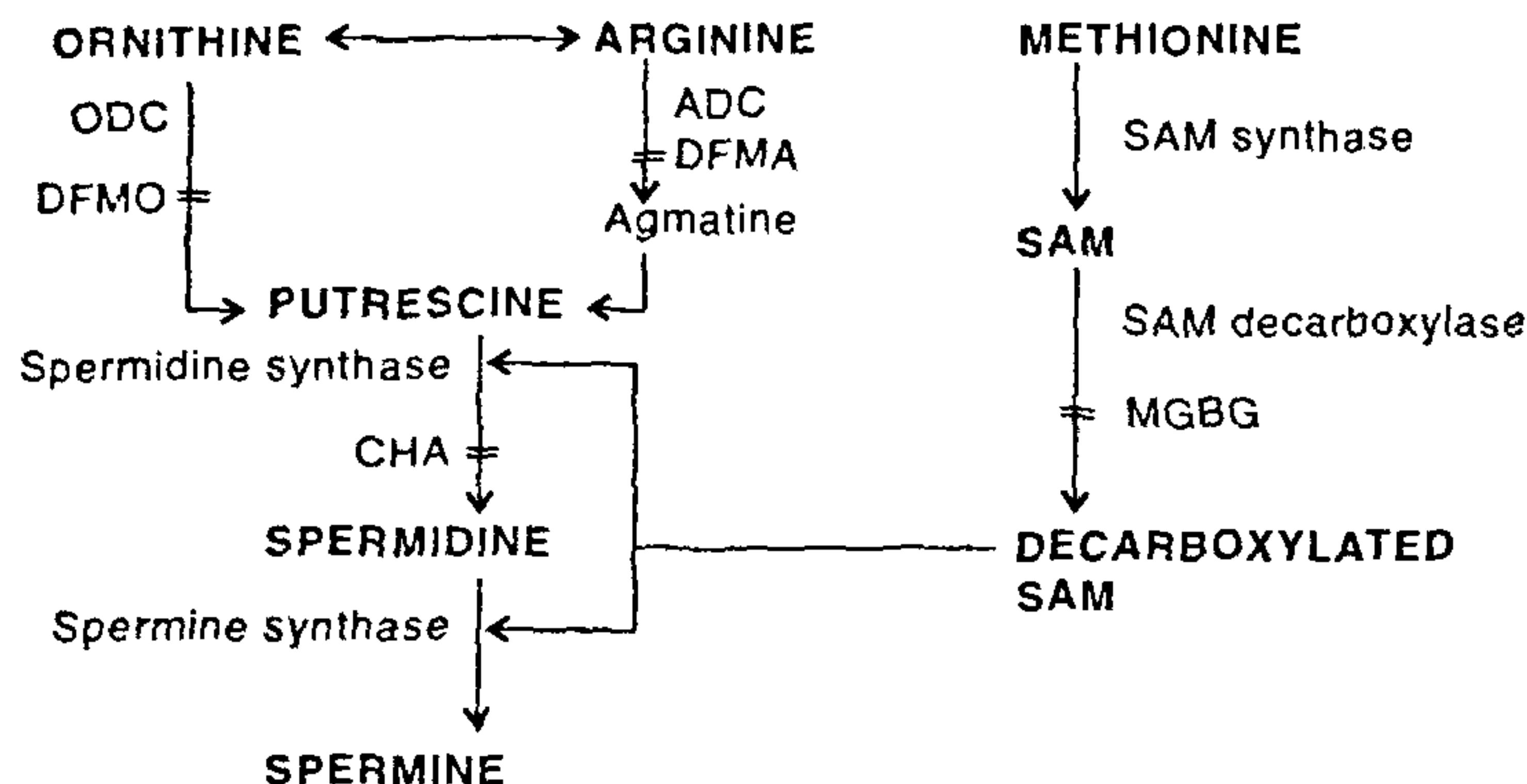


Figure 1. Polyamine biosynthetic pathway.

enzymes has provided a novel and promising approach for new therapies<sup>16</sup>, including the control of cancer<sup>13</sup> and protozoal infections<sup>11</sup>.

Rajam and Galston<sup>14</sup> and Rajam *et al.*<sup>15</sup> were the first to report the control of several plant pathogenic fungi *in vitro* and protection of pinto bean plants against bean rust fungus, *Uromyces phaseoli* using low concentration of DFMO (0.5 mM), respectively, without affecting the host plant. Further, DFMO was persistent, fast acting and translocatable<sup>15</sup>. Since then, antifungal activity of DFMO and other polyamine biosynthetic enzyme inhibitors like methylglyoxal bis (guanyldrazone) (MGBG, an inhibitor of SAM decarboxylase) and cyclohexylamine (CHA, an inhibitor of spermidine synthase) has been demonstrated by several researchers on various types of phytopathogenic fungi<sup>3,6-8</sup>. Fungal diseases controllable by DFMO include rust diseases of French bean, broad bean, wheat, oat and corn; powdery mildew diseases of wheat, barley, bean and apple; tomato wilt; corn leaf blight, and wood decay<sup>3,6-8</sup>. Among different kinds of plant fungal pathogens tested, rusts and mildews were found to be very sensitive to inhibitors of polyamine biosynthesis<sup>3,6-8</sup>. However, it has been shown that some fungi are relatively insensitive to polyamine biosynthesis inhibitors<sup>17</sup>. Further, DFMO has been shown to be inhibitory for fungal spore germination and/or sporulation<sup>7,18</sup>. Interestingly, DFMO (up to 5 mM) had no deleterious effects on polyamine biosynthesis, growth and development<sup>6-8,15,19</sup>, chlorophyll content, chromosome behaviour (both in mitosis and meiosis) and agronomically important traits<sup>19</sup>. Also,

DFMO could trigger the biochemical defense mechanism(s) in the wheat plant<sup>20</sup>. This approach has been extended to control growth and development of zoopathogenic fungi<sup>7,21</sup>, and tobacco caterpillar, *Spodoptera litura*<sup>12</sup> as well as viral infections of tobacco caused by tobacco mosaic virus and cucumber mosaic virus<sup>3,7</sup>.

Recent advancement in polyamine research has been the rapid development of molecular approaches to evaluate the roles of polyamines in plant metabolism<sup>22</sup>. Isolation and characterization of genes involved in polyamine biosynthesis, including ODC, have been reported from plants and several other systems<sup>22</sup>. The availability of these genes (both sense and antisense polyamine genes) has helped in creating transgenic plants with altered polyamine biosynthesis<sup>23</sup>, and such transgenics will be useful to study the specific roles of polyamines in plant development and stress responses. These studies also suggest that molecular approaches like antisense RNA technology could be used to selectively target fungal polyamine biosynthesis in which the action of single gene, notably ODC is knocked out for plant chemotherapy. For instance, the blockage of the expression of fungal sense ODC gene in transgenic crop plants expressing antisense fungal ODC gene (preferably with pathogen-inducible promoter) upon infection with fungal pathogens could be achieved, and this should be lethal for fungal pathogens. This antisense RNA approach may be useful for fungi that penetrate into the host plant cells. In fact, many fungi, including the most important fungi that cause rust, smut and mildew diseases

penetrate into the cells by producing haustoria<sup>24</sup>. Haustoria suck the nutrients from host plant cells, and they are referred to as specialized absorbing organs<sup>25</sup>. The absorptive nature of haustoria may facilitate the uptake of antisense mRNAs of ODC gene produced by transgenic plants, along with nutrients. This would lead to the formation of duplex RNA between sense mRNA (produced by fungus) and antisense mRNA (produced by transgenic plant), and eventually the blockage of translation of fungal ODC gene by the degradation of duplex RNA as it is very unstable<sup>26</sup>; the net result of this could be the suppression of haustoria formation or subsequent formation of secondary hyphae. Further, this antisense RNA approach may not have any adverse effect on the host plants, since they contain an alternative biosynthetic pathway (ADC), which is much more predominant than ODC pathway for polyamine formation<sup>6-8,19</sup>. The ribozyme technology may also be used for the specific blockage of ODC pathway (gene expression) in fungi for plant chemotherapy.

Antisense RNA approach may have several advantages over the use of agrochemicals for control of fungal plant infections. For instance, it may lead to reduced application of chemical pesticides, which will reduce the exposure of farmers to harmful pesticides and chemical residues in the environment. I predict that the use of polyamine biosynthesis inhibitors and probably the proposed antisense RNA approaches for the control of plant diseases of fungal origin would be one of the major interests of plant science research in the coming years and this is because of added excitement and fervour to much of the work in progress in polyamine research from several laboratories. One can only wonder if Leeuwenhoek would have ever believed that the fruits of his discovery of a polyamine would come to all of this.

1. Galston, A. W., *BioScience*, 1983, 33, 382-388.
2. Tabor, C. W. and Tabor, H., *Microbiol. Rev.*, 1985, 49, 81-99.
3. Rajam, M. V., *Plant Ecophysiology* (ed. Prasad, M. N. V.), John Wiley, New York, 1997, pp. 343-374.
4. Galston, A. W. and Kaur-Sawhney, R., *Plant Physiol.*, 1990, 94, 406-410.
5. Bey, P., Danzin, C. and Jung, M., *Inhibition of Polyamine Metabolism* (eds McCann, P. P., Pegg, A. E. and Sjoerdsma, A.), Academic Press, San Diego, 1987, pp. 1-31.

6. Galston, A. W. and Weinstein, L. H., *Progress in Polyamine Research* (eds Zappia, V. and Pegg, A. E.), Plenum, New York, 1988, pp. 589–599.
7. Rajam, M. V., *Curr. Sci.*, 1993, **65**, 461–469.
8. Walters, D. R., *Mycol. Res.*, 1995, **99**, 129–139.
9. Khan, A. J. and Minocha, S. C., *Life Sci.*, 1989, **44**, 1215–1222.
10. Zarb, J. and Walters, D. R., *New Phytol.*, 1994, **126**, 99–104.
11. Bacchi, C. J. and McCann, P. P., *Inhibition of Polyamine Metabolism* (eds McCann, P. P., Pegg, A. E. and Sjoerdsma, A.), Academic Press, San Diego, 1987, pp. 317–344.
12. Rajam, M. V., *Indian J. Exp. Biol.*, 1991, **29**, 881–882.
13. Pegg, A. E., *Cancer Res.*, 1988, **48**, 759–774.
14. Rajam, M. V. and Galston, A. W., *Plant Cell Physiol.*, 1985, **26**, 683–692.
15. Rajam, M. V., Weinstein, L. H. and Galston, A. W., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 6874–6878.
16. McCann, P. P., Pegg, A. E. and Sjoerdsma, A. (eds), *Inhibition of Polyamine Metabolism*, Academic Press, San Diego, 1987.
17. Smith, T. A., Barker, J. H. A. and Owen, W. J., *Mycol. Res.*, 1992, **96**, 395–400.
18. Khurana, N., Saxena, R. K., Gupta, R. and Rajam, M. V., *Microbiology*, 1996, **142**, 517–523.
19. Bharti and Rajam, M. V., *Ann. Bot.*, 1996, **76**, 297–301.
20. Bharti, Rajam, M. V. and Sawhney, R. N., *Phytochemistry*, 1996, **43**, 1009–1013.
21. Pfaller, M. A., Riley, J. and Gerarden, T., *Mycopathologia*, 1990, **112**, 27–32.
22. Walden, R., Cordeiro, A. and Tiburcio, A. F., *Plant Physiol.*, 1997, **113**, 1009–1013.
23. Masgrau, C., Attabella, T., Fareas, R., Flores, D., Thompson, A. J., Bestford, R. T. and Tiburcio, A. F., *Plant J.*, 1997, **11**, 465–473.
24. Cornelissen, B. J. C. and Melchers, L. S., *Plant Physiol.*, 1993, **101**, 709–712.
25. Alexopoulos, C. J. and Mims, C. W., *Introductory Mycology*, John Wiley and Sons, New York, 1988.
26. Bourque, J. E., *Plant Sci.*, 1995, **105**, 125–149.

ACKNOWLEDGEMENTS. I thank CSIR, DST and UGC, New Delhi for financial support.

MANCHIKATLA V. RAJAM

*Plant Genetic Manipulation Group,  
Department of Genetics,  
University of Delhi South Campus,  
Benito Juarez Road,  
New Delhi 110 021, India*

## Pancharatnam phase as a purely geometric phase

Any optical system designed to demonstrate the Pancharatnam phase<sup>1</sup> as a purely geometric phase must generate each new polarization state by projecting the previous state on it<sup>2</sup>. Retarders cannot be used to cycle the polarization, since they may introduce additional dynamic phases<sup>3</sup>. The only optical elements that can be used to cycle the state of polarization of the beams are analysers, where we define an analyser as an optical element that transmits the desired polarization and rejects (absorbs or reflects) the orthogonal polarization<sup>4</sup>. We describe here an interferometric arrangement for such a demonstration.

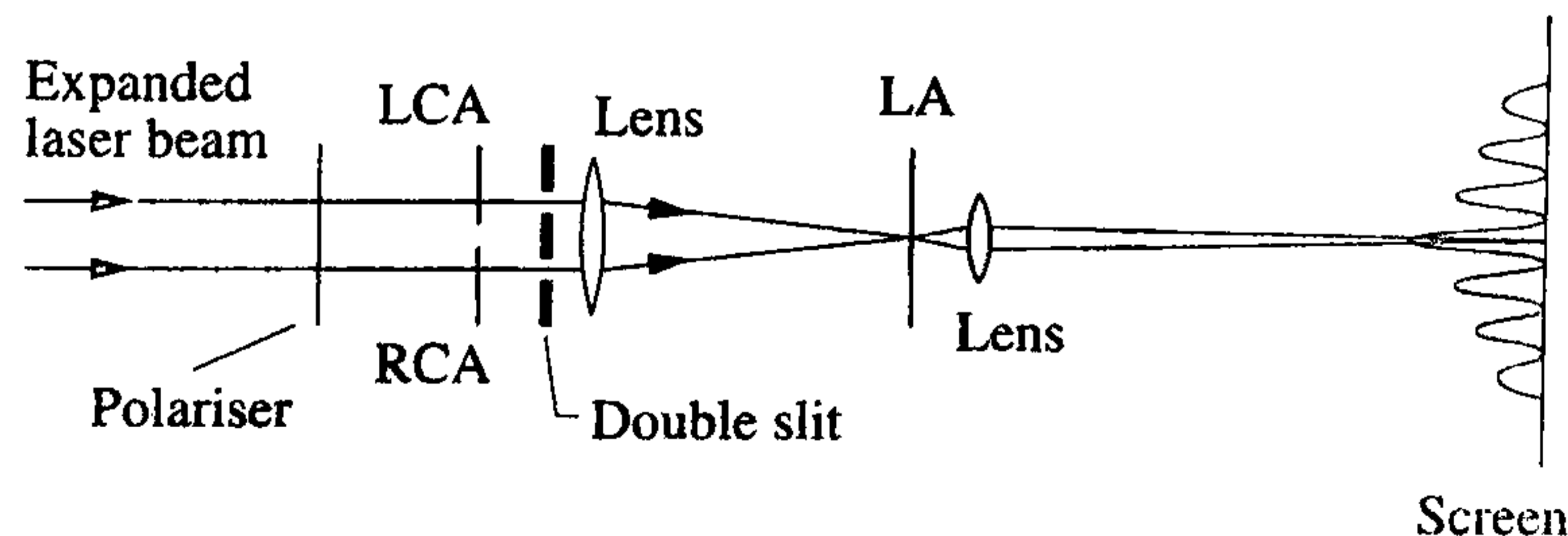
The optical system that we have used

(see Figure 1) is based on Young's interference experiment. An expanded, linearly polarized beam from a He-Ne laser ( $\lambda = 0.633 \mu\text{m}$ ) illuminates a pair of slits through two circular analysers RCA and LCA to yield, respectively, right- and left-circularly polarized states. Both these circularly polarized states are then projected by a lens on to a linear analyser LA to obtain the final linearly polarized states. A magnified image of the interference pattern produced by these two states is formed on a screen by a second lens.

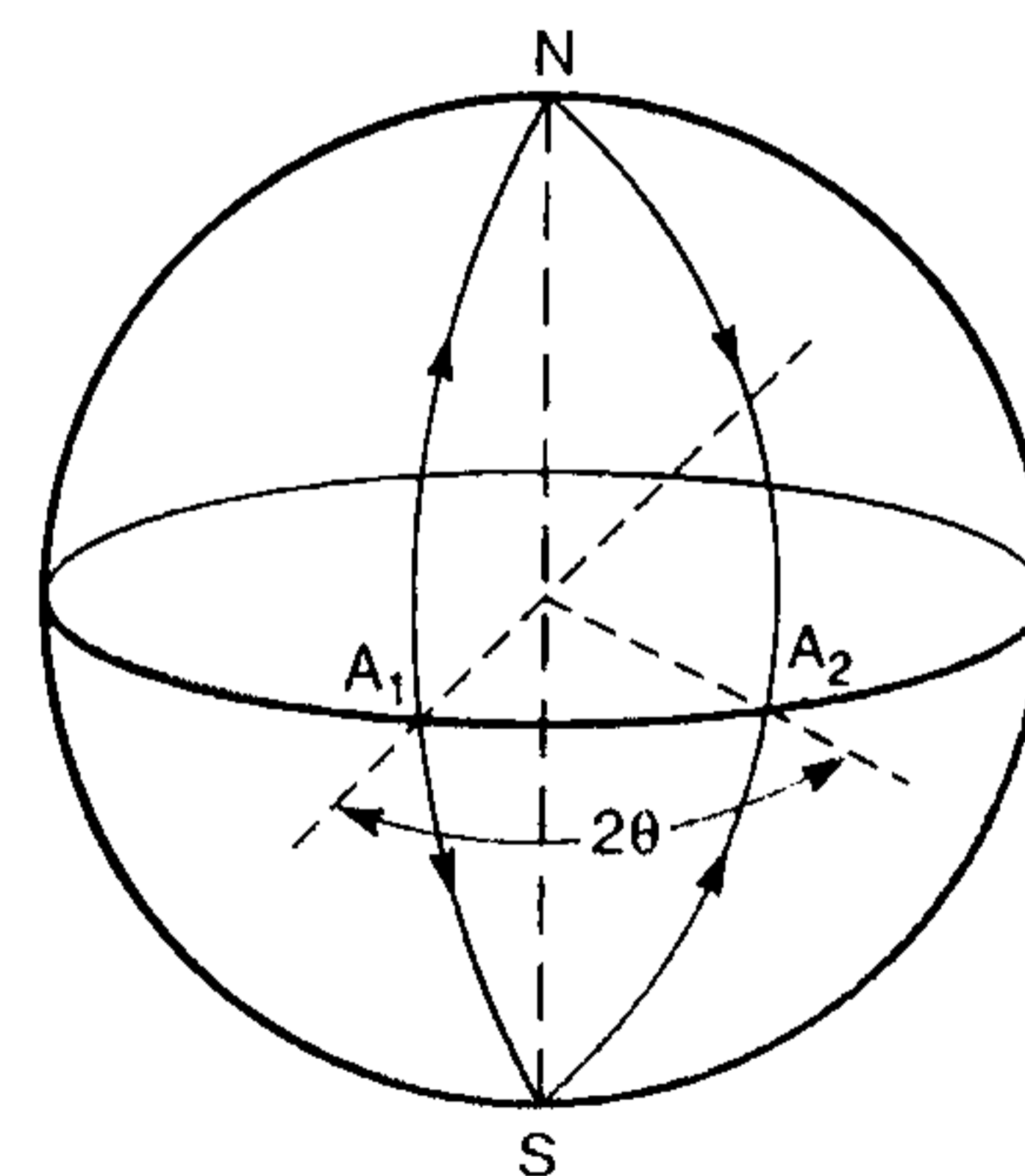
While sheet polarizers can be used as linear analysers, circular analysers, which transmit one circular polarization and

reject the other, are not readily available. We have used  $50 \mu\text{m}$  thick films of two cholesteric liquid-crystal materials<sup>5,6</sup> (76% by weight of cholesteryl oleyl carbonate in cholesteryl chloride, at  $24^\circ\text{C}$ , and 80.7% by weight of cholesteryl chloride, 17.6% of cholesteryl oleyl carbonate and 1.7% of cholesterol, at a temperature of  $38^\circ\text{C}$ ) as left- and right-circular analysers, respectively.

The operation of this system can be



**Figure 1.** Optical system of the interferometer used to demonstrate the Pancharatnam phase as a purely geometric phase.



**Figure 2.** Poincaré sphere representation of the operation of the interferometer.