Paraplegia (1973), 10, 249-255

Proceedings of the Annual Scientific Meeting of the International Medical Society of Paraplegia held at Heidelberg (Germany) on 3rd to 5th August 1972 (Part II)

EXPERIMENTAL NEUROLATHYRISM IN CHICKS

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INTRODUCTION

EXCESSIVE intake of *Lathyrus sativus* seed has been alleged to produce neurolathyrism in humans (Bell, 1964; Nagarajan *et al.*, 1965, Mani *et al.*, 1971), but the knowledge about this condition at every level is either incomplete or ill-understood, even to date. The reason for this is most probably because it has not been successfully extrapolated in the animals as revealed by the review of literature (Selye, 1957; Ganapathy *et al.*, 1963). The extent to which symptoms have been produced are of vague neurotoxic nature of short duration. Chicks are reported to be the species which manifest these symptoms, when fed *L. sativus* orally (Ganapathy *et al.*, 1963) and also monkeys when its extract was administered parenterally (Adiga *et al.*, 1963; Roy *et al.*, 1963; Nagarajan *et al.*, 1965; Mani *et al.*, 1971). Permanent symptoms of neurolathyrism in animals have been both claimed (Roy *et al.*, 1963) and denied (Nagarajan *et al.*, 1965) by the same group of workers, in the same species of animals, i.e. chicks, and by the same toxic extract which they call β-oxalylamino-L-alanine (BOAA). Thus, starting with the assumption that *L. sativus* intake is responsible for neurolathyrism the experiment about to be described was undertaken to produce the condition in chicks by both administering orally and by injecting the crude extract of *L. sativus* seeds intraperitoneally (I.P.) and to study the site of lesion and the pathological changes when produced in them.

METHODS AND MATERIALS

Fifty chicks were divided into two groups: 'Feeding' and 'Injection (I.P.)'. The animals and their stock diet (Table I) were provided by a Government of Bihar poultry farm. *L. sativus* seeds or Khesari were purchased from a local grocery store, the genuity being confirmed by both experts and laity.

Feeding. Eighteen four-week-old chicks weighing approximately 200 g. to 250 g. were divided into different batches and housed in specially improvised large cages.

A graded, coarsely ground powder of the seed was given to the experimental animals (Table II) taking care that the total digestible and crude protein levels and energy yields of the control and the experimental diets were similar. Special attention was paid to the batch kept on 100 per cent. *L. sativus*; most of this was fed in an emulsified form by means of a dropper, the dose progressively increasing from the initial 1 g. to the final 2 g. (The dropper was used to ensure that the

chicks ate the emulsion.) Routine vaccination and proper upkeep of the animals was duly supervised by expert veterinarians. This experiment lasted for seven months.

TABLE I

Control Diet

		Protein values	
		Percentage	Grams
Maize	57%	10%	5·7
Wheat bran	17%	12%	4·0
Ground-nut cake	15%	45%	6·7
Fish meal	8%	45%	3·6
Mineral mixture	3%	—	—
Vita blend	64·8 mg. in 100 Kg.	—	—
Total			20·0

TABLE II

Grading of *Lathyrus Sativus* Feed

Feed groups	Control	Voluntary feeding	Forced feeding
Stock diet with mineral and vitamins	2 chicks	—	—
50% stock diet plus 50% *Lathyrus sativus*	—	3 chicks	—
25% stock diet plus 75% *Lathyrus sativus*	—	3 chicks	—
100% *Lathyrus sativus*	—	3 chicks	—
Lathyrus sativus emulsion fed by dropper	—	—	7 chicks

Injection (*I.P.*). Thirty-two chicks of ages ranging from 1 to 8 days and weighing 38 g. to 40 g. were used for this experiment. The crude extract prepared from the same stock of *L. sativus* used in the oral feed group was injected I.P. in 26 chicks. Control fluid was injected in the remaining six, the amount varying from 0·5 ml. to 0·8 ml., as determined by trial and error method. The technique for preparing the crude alcoholic extract was basically the same as adopted by Roy *et al.* (1963) apart from some minor modifications. The control fluid was prepared similarly by using 30 per cent. alcohol, but with no seeds of *L. sativus*.

RESULTS

None of the chicks showed any neurotoxic symptoms, not even in the 'forced-feeding' batch when fed on *L. sativus* seeds for seven months continuously.

However, the new-borns of up to eight days old showed two distinctly different types of clinical neurotoxic manifestations when injected with crude extract I.P. *e.g.* short-lived and permanent ones. In the short-lived group most of the animals following I.P. injection showed drowsiness, (fig. 1) becoming alert when aroused and the slowing down of leg movements. After between 12 and 16 hours they returned to normal. In some chicks more severe symptoms such as retraction, twisting and stiffening of necks also developed and in two particular subjects the result was that they were unable to stand and so adopted a squatting

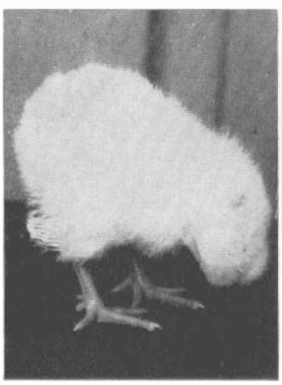

FIG. 1

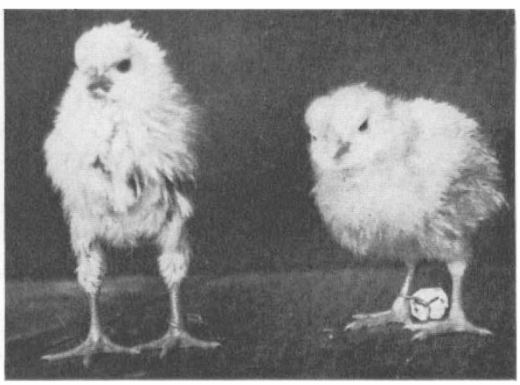

FIG. 2

Fig. 1.—Drowsiness following intraperitoneal injection of crude extract of *Lathyrus sativus* in a 5-day-old chick.

Fig. 2.—Straightened spastic legs developing in the experimental chick (*left*) following injection of crude extract of *Lathyrus sativus* intraperitoneally making it look taller than the control animal.

position: also, when handled they made backward somersaults. Chicks developing such severe symptoms as these died within 24 hours.

The most fascinating finding of the experiment, however, was that five chicks following three days of I.P. injection began standing with both legs well stiffened, almost making a straight line as opposed to their normal position which is at an angle to their knees (fig. 2). Two of these five, when slightly goaded and also voluntarily, walked with a gait likened to human struck with lathyriasis and were photographed with a cine camera. Two days later both birds died. Permanent symptoms, manifested mostly by an increasing stiffening of the legs, developed in the remaining three birds. These three were injected daily with the crude extract I.P. along with the other surviving chicks. One died after 18 days, the other after 20 and the third after 33 days. A few days prior to death the last two chicks, particularly the third, developed spastic-like legs with elicitable brisk knee jerks (fig. 3). Injection of B. Complex did not materially bring any amelioration in their symptoms. X-ray of the chicks developing permanent symptoms showed no

bony deformity not even with the most advanced symptoms. The six control chicks did not at any time show neurotoxic manifestation when injected with the specially prepared control fluid I.P. At the end of the one and a half months, the

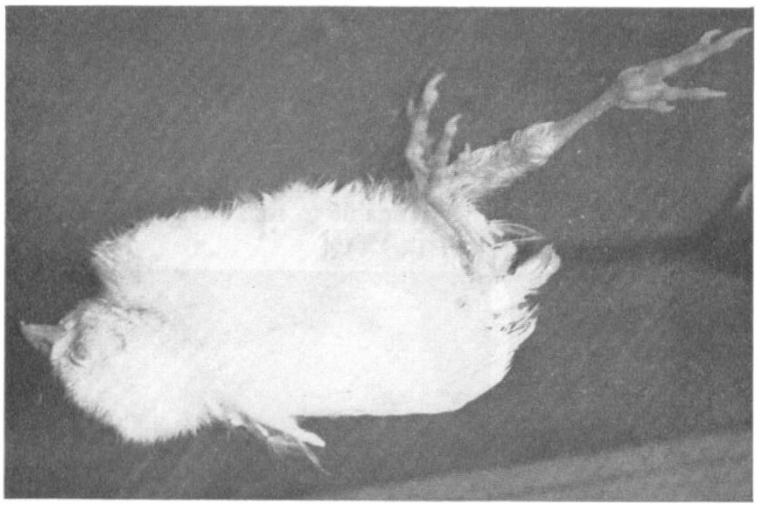

FIG. 3

Fig. 3.—Chick with permanent lesion resting on back showing the spastic left leg and the right leg photographed immediately after it elicited positive knee-jerk response.

TABLE III

Table showing break up of 32 injection group chicks symptomwise

Total No. of chicks	Groups	No. of chicks with period of death/sacrifice
32	I Control	6—Sacrificed after 1½ months
	II Experimental	
	1. Short-lived	
	(*a*) Mild	12—Died within 3-4 days
	(*b*) Severe	6—Died within 0-1-5 days
	2. Permanent	3—Died within 18-20-33 days
	3. No symptoms	5—Sacrificed after 1½ months

total survivors of the 32 chicks were; six controls and five experimental birds, which did not develop any symptoms apart from the occasional transient ones (Table III).

Histopathological changes in the central nervous system were found in most of the dying chicks in the short-lived symptom group, particularly in the two subjects which made backward somersaults; and in the three birds of the permanent symptom group which developed spasticity. In the former, the nerve cell popu-

lation of the anterior horn in the lumbo-sacral region invariably appeared appreciably less, and quite often showed an increasing degree of chromatolysis corresponding with the severity of the acute neurotoxic symptoms. In these, no white

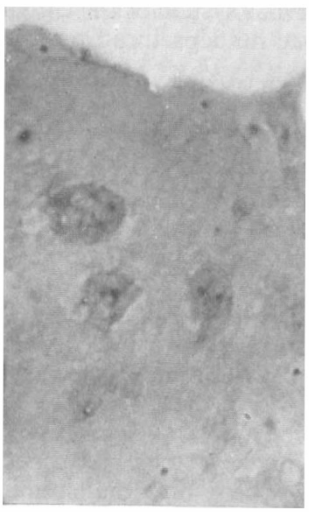

FIG. 4

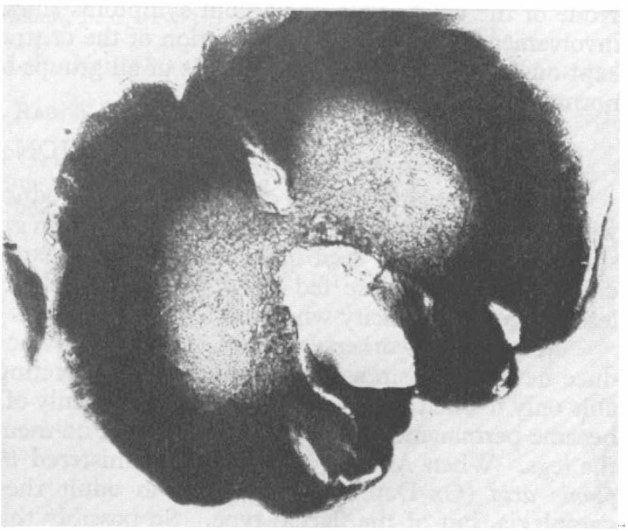

FIG. 5

Fig. 4.—Cortical area showing nerve cell degeneration. Nissl stain. × 500.

Fig. 5.—T. S. of lumbo-sacral region of a control chick. Marchi's stain. × 35.

Fig. 6.—T. S. of lumbo-sacral region of the chick with permanent symptoms showing patchy demyelinisation of the white matter. Marchi's stain. × 35.

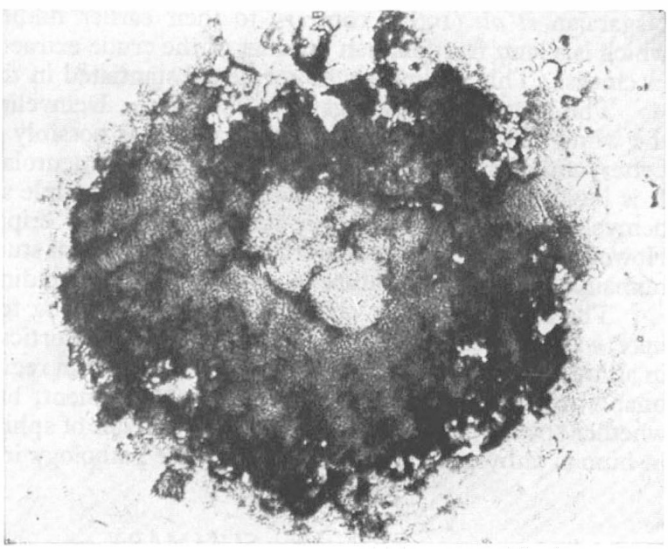

FIG. 6

matter or cortical lesions were noticeable. Of the latter group, particularly the chicks which died after 33 days, there were a few conspicuous histological changes of great significance. The first was chromatolysis of the nerve cells of a particular site which appeared to be the motor area of the cerebral cortex (fig. 4). This was,

surprisingly, identical in all three chicks of this group. Secondly, the third chick also showed chromatolysis of the nerve cells and increased glial cell proliferation in the conus medullaris. Lastly there were definite patchy areas of demyelinisation of the nerve fibres of the white matter in the lumbo-sacral region (figs. 5, 6). None of the chicks with or without symptoms showed any meningeal or vascular involvement. Histological examination of the central nervous system of the chicks kept on *L. sativus* orally and controls of all groups showed no departure from the normal.

DISCUSSION

Ganapathy *et al.* (1963) claimed to have produced *avian polyneuritis* in chicks within four to 26 days by feeding *L. sativus* seeds in graded form but none developed spastic paraplegia as seen in humans. In the experiment described none of the chicks, not even those fed by a dropper developed any neurotoxic symptoms, least to say of spasticity when fed for seven months continuously.

Most of the workers, as mentioned earlier, who tried experimentally to reproduce neurolathyrism as seen in humans by injecting the *L. sativus* extracts, were able only to elicit neurotoxicity. This was mainly of the short duration type which became permanent with higher doses. But, no mention was made of spasticity of the legs. When Mani *et al.* (1971) administered *β-N-Oxlyl-L-α, β-Diaminopropionic acid* (Ox-Dapro) intrathecally in adult rhesus monkeys they developed paraplegia, but of the flaccid type. So possibly this is the first time that spastic paraplegia reminiscent of human condition has been extrapolated in a few chicks by repeated I.P. injection of the crude extract of the *L. sativus* seeds. Secondly, Nagarajan *et al.* (1965), contrary to their earlier finding, reported that BOAA, which is a later fractionation product of the crude extract, produces osteolathyrism in chicks. This finding could not be substantiated in the present experiment.

The histological findings were revealing. Demyelinisation of nerve fibres in the white matter of the lumbo-sacral region is possibly a step ahead towards the better understanding of the pathology *vis-à-vis* neurolathyrism seen in humans. It is highly probable that neurolathyrism, like multiple sclerosis, is also one of the demyelinating diseases mainly characterised by crippling spastic paraplegia. However, there is no contemporary histopathological study, either experimental or human, available in literature to cross-check these findings.

The most intriguing observation, which is being told with some reservation here, was the visualisation of identical lesion in the cortical area, possibly the motor, in all three chicks which developed spasticity. With regard to its significance I am unable to make a definite statement at the moment, but it raises a doubt as to whether spastic paraplegia with complete absence of sphincter involvement, as seen in human lathyriasis, could be due to some pathology in the higher centres.

SUMMARY

As mentioned previously it is possibly the first time spastic paraplegia reminiscent of human lathyriasis has been produced in the chicks. Histopathological studies of such cases showed demyelinisation of the lumbo-sacral region and nerve cell degeneration in the conus medullaris and the cortical area. It is suggested that human lathyriasis especially in the absence of sphincter involvement could be due

to some lesion of the higher centres. Surprisingly, no symptoms developed in any of the chicks when fed on *L. sativus* seeds for seven months continuously.

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