# Experimental Teratogenic Lathyrism in Sheep and Further Comparative Aspects with Teratogenic Locoism

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## SUMMARY

A comparison of the gross anatomical deviations in malformed offspring from ewes that ingested the locoweed plant or a synthetic lathyrogen during gestation revealed many similarities but also certain essential differences. Similarities included abortions, contracture or overextension of the pastern joint, permanent flexure of the carpal joint, lateral rotation of the forelimbs, osteoporosis and bone fragility, and brachygnathia. Features restricted to the lathyrogen-induced condition included spinal malformations of various kinds especially scoliosis but occasionally kyphosis and torticollis, and forelimb abduction resulting from loose scapular attachment. The loose or hypermobile stifle joint of the loco-induced condition was rare in malformed offspring from lathyrogen-fed animals.

## RÉSUMÉ

La comparaison des déviations anatomiques macroscopiques observées chez les agneaux difformes issus de brebis ayant ingéré de l'astragale ou un lathyrogène synthétique durant la gestation, a révélé plusieurs ressemblances mais aussi certaines différences essentielles. Les ressemblances étaient les suivantes: avortements, contracture ou extension excessive du paturon, courbure permanente du genou, rotation latérale des membres antérieurs, brachygnathie, ostéoporose et fragilité osseuse. Le lathyrisme se caractérisait par différentes malformations de la colonne vertébrale, entre autres de la scoliose, parfois de la cyphose et du torticolis, ainsi que par une abduction des membres antérieurs consécutive à un attachement incomplet de l'épaule. La mobilité excessive du jarret, consécutive à l'ingestion d'astragale, était rare chez les agneaux difformes issus de brebis ayant ingéré le lathyrogène.


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## INTRODUCTION

Osteolathyrogens interfere with formation of covalent cross-links in collagen and elastin (1, 10). Consequences of ingestion of osteolathyrogens during gestation include congenital defects (3,11,12,13) and fetal resorption (14). The teratogenic effects center in tissues in which collagen or elastin are found or in which they play a developmental role (12) and result from failure of normal cross-link production.

We have been interested in the similarities in sheep between teratogenic effects resulting from ingestion of osteolathyrogens and effects resulting from ingestion of certain members of the *Astragalus* genus (locoweed plants). We have reported some preliminary observations on these similarities (16) and preliminary results suggesting the possible presence of lathyrogens in locoweed (6). We have also communicated details on the loco-induced congenital defects (4,5). In many ways, the latter resemble defects reported in the literature (12) on naturally occurring or experimental osteolathyrism in many animal species.

Common osteolathyrism effects reported in the literature include bone fragility, exostoses, long bone curvature, poor connective tissue development, kyphosis, scoliosis, osteoporosis (3,11,12,13,14). Congenital effects in locoism (4,5) include abortions, embryonic death, brachygnathia, small and weak lambs at birth, either contracted or overextended pastern joints or both, contraction of the metacarpal joint, twisted or bowed limbs, lateral rotation of forelimbs, and loose stifle joints.

Little detail is found in the literature on naturally occurring or experimental osteolathyrism in sheep especially congenital effects. This study was undertaken to more fully elucidate teratogenic lathyrism in sheep and compare it with teratogenic locoism. We investigated dose requirements, gestational chronology, and nature of gross lathyritic effects in teratogenic lathyrism of sheep induced by ingestion of the synthetic lathyrogen aminoacetonitrile (AAN) and compared them with literature reports, and experimental and field cases of congenital locoism.

## MATERIALS AND METHODS

Three groups of sheep were compared: A) one group fed AAN, B) one group fed *Astragalus lentiginosus* and C) a group of controls fed neither.

### GROUP A

The AAN dosing schedule by periods of gestation is shown in Table I. One lot of 24 sheep was divided into four groups of six and each animal was given 0.6 gm AAN/day orally in a capsule during a 30 day period of gestation. Another lot of 33 was divided into 11 groups of three and each animal given 1.5 gm AAN/day for a ten day period of gestation. A third lot was divided into four groups of three and each animal given 2 gm AAN/day for a 30 day period during gestation. All sheep were given alfalfa hay, salt and water free choice. The ewes were exposed to the ram daily throughout the early part of the experimental period. The first day the ewe accepted the ram was counted as day zero. Any ewe that did not recycle after a 20 day period was counted as pregnant. The ewes were observed daily throughout the gestation period for signs of poisoning and abortions. Lambs were examined at birth for abnormalities. Examinations were continued until it was determined if deformities were permanent. A few were maintained for periods up to a year to determine the effect of deformity on survival.

### GROUP B

Lambs born to ewes fed AAN were compared to those born to ewes that had been fed locoweed (*Astragalus lentiginosus*) at a level of 340 gm (dry weight)/day by stomach tube (4,5) during similar periods of gestation. The locoweed was collected and processed as previously described (5). The ewes receiving locoweed were fed and housed in a manner similar to those receiving AAN.

### GROUP C

The lambs born to ewes fed locoweed or AAN were compared with some 200 other lambs born on these premises during the same period to ewes that had not been fed locoweed or AAN. The latter lambs served as untreated controls and ruled out infectious disease or endogenous nutritional factors as being responsible for the effects observed.

Selected lambs were photographed, and radiographs were made of defleshed bones.

Osteoporosis was assessed by a comparison of radiographs, spontaneous fractures observed and the general fragility of the bones or resistance to shearing or breaking.

Observations on the spinal abnormalities were gross observations of animals, radiographs and defleshed spinal columns.

## RESULTS

High dose levels of AAN in dams increased the incidence of abortions, while malformations were more common at low-dose levels (Table I). Administration of AAN at the high dose level for ten days or at the low dose for 30 day periods caused

death in some of the ewes. At the low-dose level, abortions were most common when AAN was given during the first trimester and to a lesser extent the third trimester of pregnancy. Malformed offspring were more frequent the second trimester. Malformations were induced by feeding AAN for as few as ten days at any period from the 20th to 129th day of gestation.

Contracture or overextension of the pastern joint, permanent flexure of the carpal joint, and lateral rotation of forelimbs were common both in offspring from AAN-fed ewes (Fig. 1 and Table I) and in offspring from locoweed-fed ewes (4,5) (Fig. 2).

Osteoporosis and bone fragility (note fractures Fig. 3) were common and severe in offspring from AAN-fed ewes and moderate in offspring from locoweed-fed ewes. The bones of affected lambs were more easily cut or broken when compared to normals. Metacarpals, metatarsals, and skull bones from lambs born to ewes fed AAN especially were fragile and soft.

Front limb rotation in offspring from

**TABLE I. The Effect on Lambs From Oral Administration of AAN to the Dams During Gestation**

| Period of Gestation Fed AAN | No. Ewes | Total No. Lambs | Normal Lambs | Affected Lambs | Aborted | Dead at Birth | Flexure of Carpal Joint | Contracted Pastern | Overextended Pastern | Spinal Abnormality | Twisted or Bowed Limbs | Lateral Rotation of Forelimb | Loose Stifle | Brachygnathia |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0.6 gm AAN for 30-day periods** | | | | | | | | | | | | | | |
| 40—70 | 6(1)[a] | 4 | 2 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 60-90 | 6(1)[a] | 10 | 4 | 6 | 0 | 0 | 5 | 5 | 1 | 0 | 4 | 1 | 0 | 0 |
| 80-110 | 6 | 13 | 7 | 6 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 2 | 0 | 0 |
| 100—130 | 6(2)[a] | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | 24 | 32 | 18 | 14 | 2 | 1 | 9 | 6 | 2 | 0 | 6 | 3 | 0 | 0 |
| **1.5 gm AAN for 10-day periods** | | | | | | | | | | | | | | |
| 20—29 | 3 | 5 | 2 | 3 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 30—39 | 3 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40—49 | 3 | 3 | 0 | 3 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 |
| 50—59 | 3 | 4 | 0 | 4 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 |
| 60—69 | 3 | 7 | 1 | 6 | 0 | 1 | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 0 |
| 70—79 | 3 | 6 | 0 | 6 | 0 | 2 | 4 | 3 | 1 | 1 | 3 | 0 | 0 | 0 |
| 80—89 | 3 | 6 | 1 | 5 | 0 | 1 | 2 | 1 | 0 | 4 | 2 | 2 | 0 | 0 |
| 90—99 | 3 | 4 | 1 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| 100—109 | 3 | 6 | 5 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 110—119 | 3 | 5 | 3 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 120—129 | 3 | 6 | 3 | 3 | 1 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Totals | 33 | 55 | 16 | 39 | 9 | 8 | 13 | 6 | 2 | 10 | 15 | 2 | 1 | 0 |
| **2 gm AAN for 30-days periods** | | | | | | | | | | | | | | |
| 40—70 | 3(2)[a] | 4 | 0 | 4 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60—90 | 3 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 80—110 | 3 | 5 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100—130 | 3(1)[a] | 5 | 0 | 5 | 2 | 0 | 2 | 0 | 0 | 1 | 3 | 0 | 0 | 2 |
| Totals | 12 | 17 | 0 | 17 | 13 | 1 | 2 | 0 | 0 | 1 | 3 | 0 | 0 | 2 |

[a]Numbers in parentheses indicates ewes that died from the experimental treatment

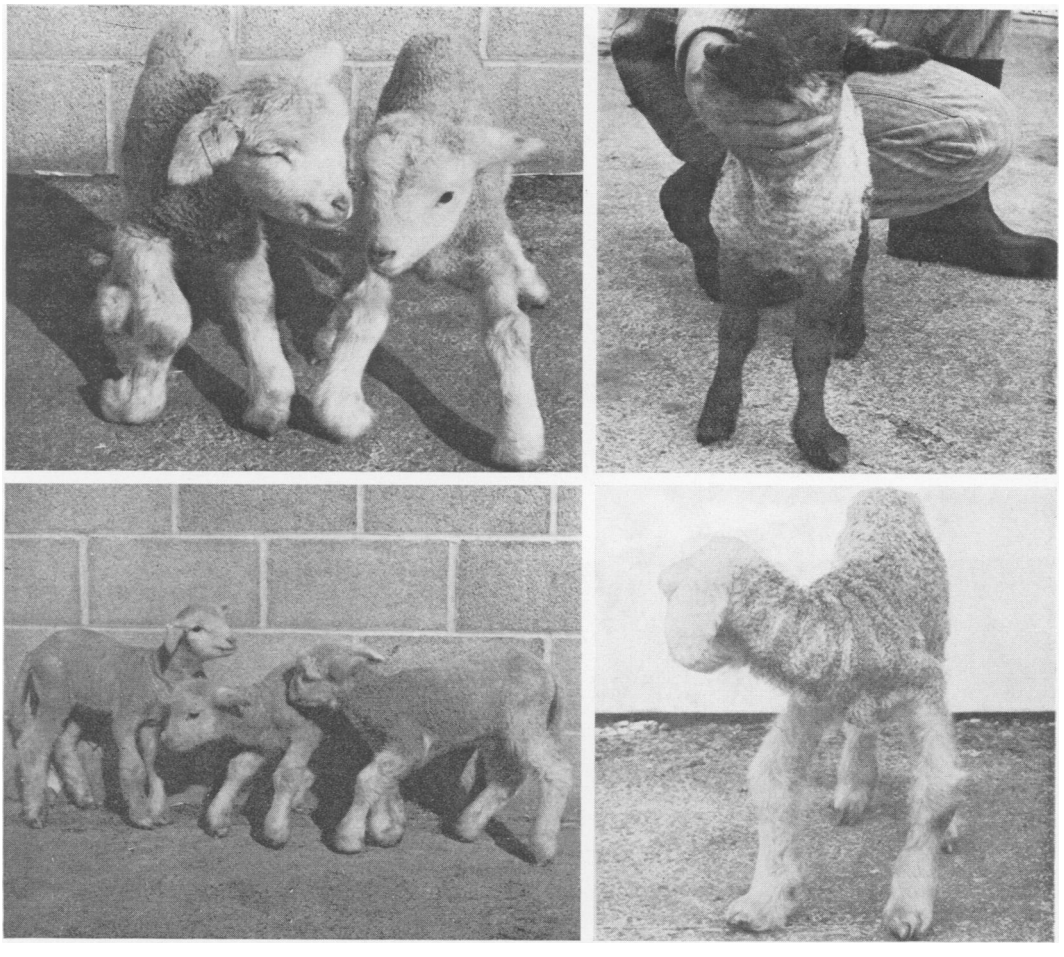

Fig. 1. Photographs of lambs with the common congenital malformations induced by maternal consumption of AAN showing contraction (1A) and overextension (1B) of the pastern-fetlock joint, permanent flexure of the carpal joint (1C), and lateral rotation of the forelimbs (1D). The dams of these lambs were fed AAN during the following gestation periods: (1A) 60 to 69, (1B) 60 to 90, (1C) 70 to 79, (1D) 90 to 99.

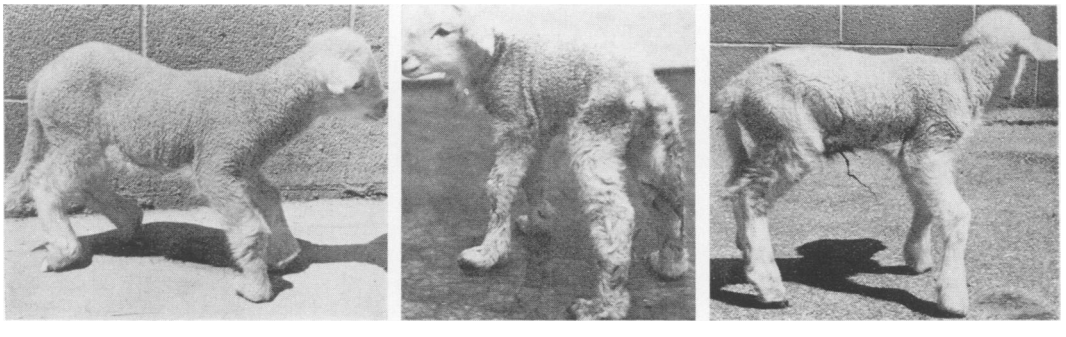

Fig. 2. Photographs of lambs with the common congenital malformations induced by maternal consumption of locoweed showing contraction (2A) and overextension (2B) of the pastern-fetlock joint, permanent flexure of the carpal joint (2C) and lateral rotation of the forelimbs (2B). The dams were fed Astragalus lentiginosus during the following periods of gestation: (2A) 40 to 70, (2B) 80 to 100, (2C) 25 to 49.

**Fig. 3. Photographs and X-rays of lambs and lamb limbs from animals born to AAN-fed dams showing osteoporosis, general fragility of limb bones and suggesting poor development of tendons and ligaments of joints. The lambs were born to ewes fed AAN from the 60 to 80 days (Fig. 3A) and 100 to 130 days of gestation (Fig. 3B).**

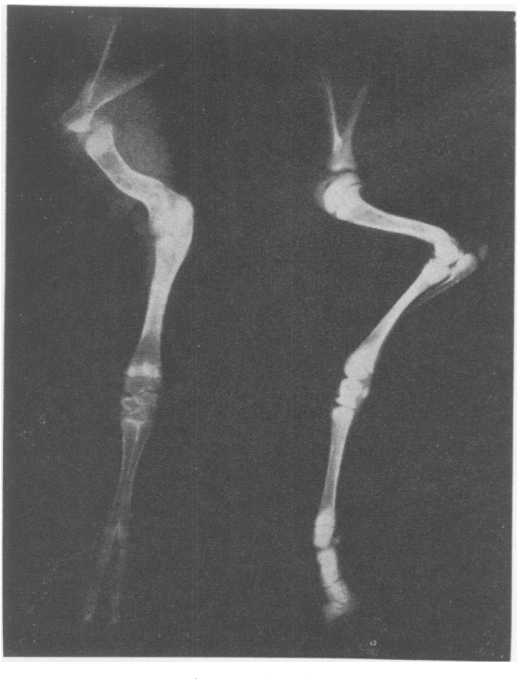

**Fig. 4. Marked rotation of the shafts of the tibia and humerus of the front limb of a lamb (on the left) born to a ewe fed AAN during the 100 to 130 days of gestation. Compared with a normal on the right. Note the bend in the humerus of the AAN-fed animal and note also thinness of the bone cortex compared with the normal. The difference in density was probably not as great as the radiographs suggest since the normal is a lateral shot while the abnormal is mostly an A/P shot because of rotation.**

AAN-fed ewes appeared to result in some cases from actual rotation of the shafts of long bones (Fig. 4) and in other cases from joint malalignment as a consequence of malocclusion of the articular surfaces of the joints.

Brachygnathia was present in both conditions but was not common. Spinal malformations of various types were found in lambs born to ewes fed AAN but were absent in lambs born to ewes given locoweed. Congenital scoliosis was most common, but kyphosis and torticollis were sometimes also seen. The immediate cause of the scoliosis was generally a wedging of the thoracic vertebrae wherein the affected vertebrae developed assymetrically in wedge shape. Lambs with AAN-induced congenital scoliosis did not recover with age. Severe examples seldom survived, and those that did could not ambulate properly. Those of mild severity survived and developed compensating ambulatory abilities. Scoliosis of this type was not observed in congenital locoism. Loose stifle joint (hypermobility) was common in the loco condition (4,5) but rather rare in lambs from ewes given AAN although forelimb abduction resulting from a weak scapular attachment was seen and recorded arbitrarily under twisted or bowed limbs in Table I to distinguish it from loose stifle.

Carpal flexure (buck knee), as in locoism, was the most commonly seen aspect of teratogenic lathyrism (7). Mildly affected lambs recovered in both cases. In severe flexure, however, malformation of the bones of the carpal joints and malocclusion of the articular surfaces of the joint prevented recovery. Deformed lambs, which did not recover, were able to survive when given proper care, but the leg and back deformities became more severe with increased age and weight. Exostoses (2,9) around deformed joints, particularly the carpal joint,

were rarely seen in offspring at birth from either AAN or locoweed-fed ewes, but were common some months later and evidently as a process secondary to the original malformation.

## DISCUSSION

In assessing whether the teratogenic aspects of locoism and lathyrism are identical, a number of comparisons must be explored; the clinical or gross morphology of the lesion, whether compounds of similar class are responsible, whether similar treatment will prevent both, and whether identity can be established in the pathological and biochemical aspects of both maladies. We have considered the first of these in experiments reported here.

The data reported here show many similarities between the teratogenic and abortifacient consequences in locoism and lathyrism. The differences are equally striking. One should be reminded, however, that not all lathyrogenic plants (retrospectively known to contain different lathyrogens) produce identical clinical effects in animals of the same species (12), even though their effects appear to result from a similar mechanism. Thus, the differences seen here between locoism and lathyrism cannot exclude the possibility that both occur by a similar mechanism. Taken alone, however, the data cannot unequivocally incriminate a lathyritic mechanism in the loco effect.

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