Experimental Lathyrism. (19785)

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Previous reports from this laboratory have been concerned with experimental lathyrism in rats and mice(1,2). We now report additional investigations with 3 of the species of *Lathyrus* previously studied (*L. odoratus, L. sylvestris Wagneri,* and *L. latifolius*), and with 2 new species,† *L. splendens* (the campo pea) and *L. strictus*. Preliminary studies of the concentration of the noxious principle of *L. sylvestris Wagneri* are reported. This species in our experience has shown itself to be the most toxic of all the species of *Lathyrus* studied.

The diets and general plan of the experiments were the same as those of earlier studies (1,2). The ground peas comprised 50% of the diet with 10% of casein included to provide an adequate amount of an animal protein of good quality.

The seed of *L. splendens* was found to be of about the same toxicity for white rats as *L. latifolius*(2). Four rats with an average initial weight of 92 g died in 5 to 11 days after consuming an average of 15 g of the legume. The symptoms produced, however, were quite different in that no hyperirritability or convulsive seizures so characteristic of rats fed *L. sylvestris Wagneri* or *latifolius* were noted.

The experiments with *L. strictus*‡ were limited by the small amount of the seed of this species available. Three rats of approximately 120 g initial weight consumed 112, 104 and 115 g of the diet respectively over a period of 14 days (equivalent to approximately 55 g of the legume). During this period the changes in weight were −3.5, −13.9, and +2.3 g as compared with +11.7, +2.5 and +19.7 g in pair-fed litter mate controls which received the edible white split pea of commerce. No evidence of toxicity was observed, apart from the failure to gain as did the control rats. It is evident that any toxicity of *L. strictus* was definitely less than that of *sylvestris Wagneri, splendens,* or *latifolius*. However, it was more toxic (as shown by losses in weight) than *odoratus, sativus,* or *hirsutus*(1).

TABLE I. Relative Toxicity of *Lathyrus* Legumes for White Rat and White Mouse.

Lathyrus species	Fatal dosage*	Relative toxicity†
Rat		
sylvestris Wagneri	5.8	100
splendens	20.7	28
latifolius	22.3	26
odoratus	289	2
Mouse		
sylvestris Wagneri	25.4	100
latifolius	32.8	78

* Amount of pea per 100 g body wt which produced death.

† The toxicity of *L. sylvestris Wagneri* is arbitrarily designated as 100.

TABLE II. Toxicity of *L. sylvestris Wagneri* and *L. latifolius* to Different Species.

	No. of animals	Fatal dosage*	Ratio with the rat as unity
Lathyrus sylvestris Wagneri			
Guinea pig	3	3.3	.57
Rat	14	5.8	1
Rabbit	3	6.7	1.15
Mouse	3	25.4	4.37
Lathyrus latifolius			
Rat	4	22.3	1
Mouse	3	32.8	1.47

* Amount of pea per 100 g body wt necessary to produce death.

A comparison of the toxicities of the 4 chief species of *Lathyrus* studied on the basis of the amount of the legume eaten per 100 g of body weight is given in Table I. It is seen that *L. sylvestris Wagneri* is 3 to 4 times more toxic than *splendens* or *latifolius* and about 50 times more toxic than *odoratus* for the rat.

Intoxication due to *L. sylvestris Wagneri* was also studied in other species of animals

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† Seed of *Lathyrus strictus* was obtained from Mrs. E. W. Cisler of San Diego, Calif., and of *L. splendens* from Theodore Payne of Los Angeles with the cooperation of Mrs. Cisler.

‡ These experiments were conducted by Mr. T. R. Sawyer as a research assistant.

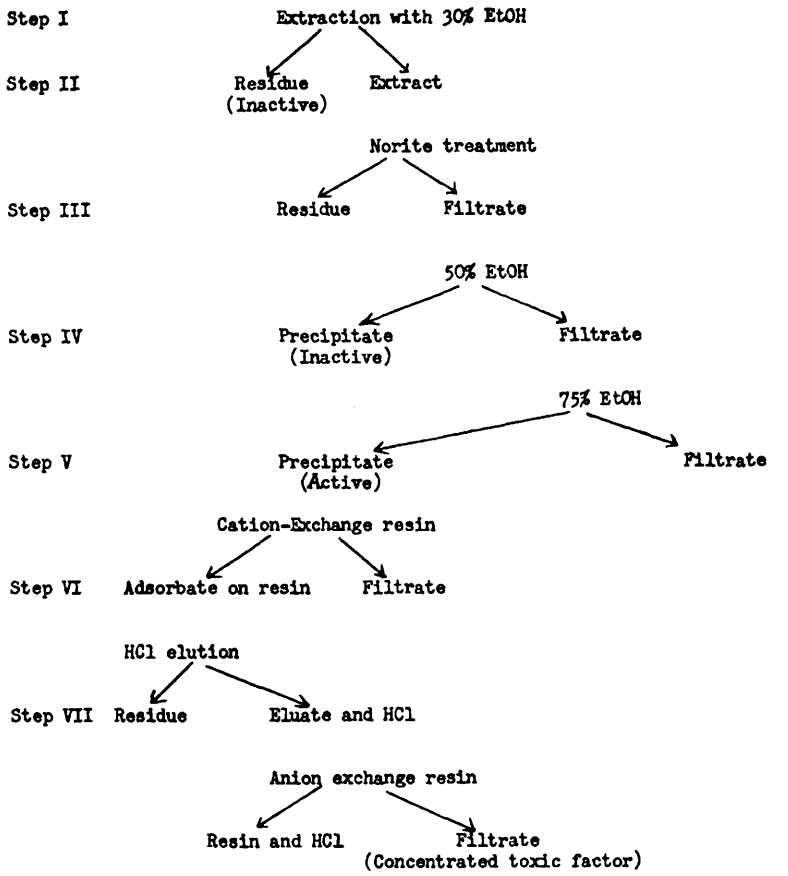

FIG. 1. Scheme of fractionation.

(Table II). It is known that there is a great species variation of toxicity in animals receiving *Lathyrus*, notably among domestic animals (3). Essentially the same amount of legume per 100 g of body weight was required as the fatal dose in the rabbit as in the rat. Death occurred when the guinea pig had consumed only 60% of this amount, while 4 times this amount of *sylvestris Wagneri* was required to produce fatal toxicity in the mouse. The mouse also proved more resistant to intoxication by *latifolius* than the rat (Table II).

A procedure, devised for the concentration of the toxic factor in *L. sylvestris Wagneri*, is detailed to Fig. 1. Step I is the extraction of the toxic principle from the finely ground legume with 30% ethanol, as previously reported(1), an extraction which removed approximately 20% of the solids. This extract is dried *in vacuo* at 50 to 60°, incorporated into a 10% aqueous solution, mixed with Norite (10 g per 1 of solution), shaken for one hr, and the solution is filtered. About 35% of the solids are removed with the Norite; the toxic principle remains in the filtrate. In step III, an equal volume of ethanol is added to this filtrate, which results in the precipitation of a small amount on an inactive fraction (less than 1% of the total solids). When the concentration of ethanol is increased to 75%, approximately half of the total solids is precipitated; this fraction contains the greatest part of the toxic principle. After filtration, the precipitate is dissolved to give a 2% aqueous solution and passed through a cation-exchange column.§ The resin

§ The Rohm and Haas cation-exchange resin IR-100-H AG was employed. The recommendations contained in their laboratory manual were followed. Columns of 22 mm internal diameter and 120 cm long filled to a height of approximately 70 cm with 150 g of resin were used.

TABLE III. Concentration of Toxic Factor of *Lathyrus sylvestris Wagneri.*

No. of rats	Avg wt, g	Treatment	Fatal dosage/100 g rat, g	Concentration	
				In single step	Over all
14	58.7	None	5.8	—	—
4	61.4	Extn. with 30% EtOH	1.8	3.22	3.22
3	76.2	Norite treatment	.85	2.12	6.82
6	57.8	EtOH pptn. Column absorption and elution	.49	1.73	11.84
4	51.4	2% acid elute	.35	1.40	16.6
4	53.1	4% " "	.145	3.38	40

adsorbs about 60% of the solids from the solution; the toxic principle is contained in the adsorbed fraction. Step VI involves the elution of the adsorbed material by hydrochloric acid. The separation and concentration is completed by the removal of the acid by ion exchange resin IR-4B.

The acid eluates from Step VI were found to vary in toxicity, depending upon the amount and strength of the acid used. Weak acid (1% or less) elutes material of low toxicity, whereas subsequent elution with stronger acid (2-4%) gives highly toxic fractions. For example, in one run, 15 g of material was adsorbed onto the resin column (Step V) and serially eluted with approximately 1 l each of 0.5, 1, 2, and 4% hydrochloric acid. The weakest acid (0.5%) eluted 8 g from the column, while the 3 higher concentrations eluted about 2.3 g each. The amounts of each fraction which effected the death of young white rats (approximately 60 g in weight) were 0.68, 0.32, 0.19 and 0.08 g respectively.

Toxicity of each fraction was assayed by feeding to the rat with the basal white pea diet and determining the fatal dosage. The concentration of the toxic factor which has been effected at each step in a typical experiment is shown in Table III. In these data the fatal dose is calculated in terms of 100 g body weight.

A total of 10 species of *Lathyrus* has now been studied in our laboratory in relation to the nutritive value and toxicity of these leguminous seeds for the young white rat. Of these, *Lathyrus sativus* and *cicera* (reported to be of clinical importance in the lathyrism of man) proved to be relatively non-toxic to the young white rat and supported moderately good growth over considerable periods of time, growth comparable with that observed with *Pisum sativum,* the edible dried pea of commerce. On the other hand, the toxicity of *sylvestris Wagneri* was greatest of all the species of *Lathyrus* examined; this was true for all the species of animals to which it was fed (white rat, white mouse, rabbit, guinea pig). Of the other species, *latifolius* and *splendens* were extremely toxic, as was also *tingitanus* to a slightly lesser degree. Experimental lathyrism with marked changes in bones(1) and related structures was readily produced in 6-12 weeks with *odoratus* and in approximately 16 weeks with *hirsutus.* It would seem highly improbable that the marked skeletal changes observed with these 2 species would be produced by species which were so toxic as to induce death within a few days (*sylvestris Wagneri, latifolius, splendens*). The toxicity of the first 2 of these was associated with characteristic nervous symptoms (marked irritability and convulsion) which became progressively more acute until death ensued. There were slight variations in toxicity in relation to the species of animal, but with one exception the differences were not marked. However it was not possible to observe the toxicity and skeletal changes in mice fed *odoratus* such as were seen in rats.

The nature of the toxic principle of the various species of *Lathyrus* is not known. In all the species which we have studied, extraction with cold water left a relatively nontoxic residue; ether failed to remove the toxic agent. For practical purposes, we have found that 30% alcohol is the most effective extractant. From such an alcoholic extract of *Lathyrus sylvestris Wagneri,* we have been

able to effect an approximately 40-fold concentration of the toxic material by absorption on a cation-exchange resin, from which the toxicant could be eluted by 2-4% hydrochloric acid. In view of the interest in lathyrism, experimental and clinical, we are reporting these observations, since there is no prospect that we can extend our investigations of this subject in the immediate future.

Summary. A comparison of the toxicity of the seed of 5 species of *Lathyrus* for the rat has been made. Intoxication with *L. sylvestris Wagneri* has also been studied in the white mouse, rabbit, and guinea pig. A partial purification of the toxic principle of *L. sylvestris Wagneri* which has concentrated the factor about 40 times is described.

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Renal Transport of Para-Aminohippurate in the Hypophysectomized Rat.* (19786)

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A number of investigators have shown that the clearance of inulin, diodrast and para-aminohippurate as well as the tubular transport maximum (Tm) of para-aminohippurate and glucose are reduced in hypophysectomized animals(1-5). The reduction in Tm as determined with para-aminohippurate (PAH Tm) could be due to a reduction in the renal blood flow or due to a defect in the tubular transport of para-aminohippurate. The latter point can be readily studied by the para-aminohippurate concentration technic of Cross and Taggart(6) in kidney slices.

Methods. Male, Sprague Dawley rats, 30-36 days following hypophysectomy† were used in this study; litter mates were used as controls. The hypophysectomized rats weighed 89 to 97 g while the control litter mates weighed 110 to 230 g. A series of hypophysectomized rats were injected with the equivalent of 1 mg of growth hormone‡ (Armour standard) per 100 g of body weight twice daily for 5 days. Two growth hormone preparations (Armour & Co.)‡ prepared by the method of Wilhelmi, *et al.*(7) were administered to each of 2 groups of hypophysectomized rats. Kidney slices were prepared by conventional methods and incubated in a buffer similar to that of Cross and Taggart(6). The PAH concentration used was 0.0013 molar. The weight of the incubated slices varied between 55 to 80 mg. One set of vessels contained no substrate while the other set contained 0.01 molar sodium acetate. The vessels were incubated for 2 hours at 25°C in an oxygen atmosphere in a Dubnoff metabolic shaking incubator. PAH determinations in the slice and medium were determined by the same methods as those used by Cross and Taggart(6).

Results. Cross and Taggart have expressed the PAH concentrating ability of kidney slices as the slice medium PAH concentration ratio (SM ratio). In the present series of experiments, the SM ratio will be used as an index of activity of the renal slices.

Table I summarizes our results obtained so far and it is clear that the kidney slices from hypophysectomized rats have a much lower SM ratio than slices from control rats. The administration of growth hormone for 5 days increased the body weight of the rats by about 24 grams and the para-aminohippurate SM

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† Hypophysectomized rats were supplied by Hormone Assay Laboratory, Chicago, Ill.

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