VOLUME XXXIV JUNE, 1934 No. 2

THE CHEMISTRY AND PHARMACOLOGY OF LATHYRUS PEAS

BY RALPH STOCKMAN, M.D.

Professor of Materia Medica and Therapeutics, University of Glasgow

IN previous papers I have discussed lathyrus poisoning in animals and the disease lathyrism in man, and concluded that they are due to a poison present in the peas and have no connection with the so-called "deficiency" diseases[1]. The poison was described as an acid readily extracted by water and precipitable from it by strong alcohol or lead acetate. From the lead precipitate by further treatment a practically pure sodium salt was obtained which proved very toxic and when given hypodermically to monkeys produced acutely the same nerve lesions and the same symptoms as prolonged feeding with lathyrus peas. It was also toxic to rabbits and frogs. The acid body was not identified at the time, but later investigation showed it to be an acid salt of phytic acid. Widely spread degenerative changes were found in the nervous system[2]. Further research has put the whole matter in a clearer light, and I now propose to give a more detailed account of the chemistry of the peas and of the toxic actions of certain substances which can readily be obtained from them. Similar active bodies are obtainable from all cereal and leguminous seeds, and the results have, therefore, a wide and general bearing.

The composition of lathyrus peas is much the same as that of many other pulses but varies within certain narrow limits according to differences in soils and seasons. Undecorticated seeds grown in India gave the following figures:

	%		%
Water	10–13	Fibre	4·4–5·6
Albumen	23–32	Fat	0·84–1·7
Starch	53–55	Ash	1·95–3·5
Sugar	2·30		

The composition of the ash is of interest as showing the relatively large amounts of phosphorus and potassium present. Four samples of undried undecorticated peas were ashed and the principal constituents determined quantitatively with the following results:

	I	II	III	IV
	%	%	%	%
Total ash	2·7	3·5	1·95	2·1
Soluble in water	62	44	66	66
Insoluble	38	56	34	34
Silica and silicates	5·0*	15·0	1·0	1·0
P_2O_5	28·0	22·0	28·0	26·0
Potassium	29·0	29·6	—	—
Sodium	2·3	1·8	3·1	3·1
Calcium (CaO)	9·0	9·0	8·0	10·0
Magnesium (MgO)	9·3	8·2	8·6	9·0
Iron (Fe_2O_3)	0·7	1·74	0·3	0·3

* Per cent. of ash.

[1] *Edin. Med. J.* 1917, Nov.; *J. Pharm. and Exper. Therap.* 1929, **37**, 43; *J. Hygiene*, 1931, **31**, 550; *Janus*, 1932, 180.

[2] *J. Hygiene, loc. cit.* and 1933, **33**, 204.

The peas contain no inorganic phosphates, the phosphorus being present entirely in organic form as phytic acid combined with the various bases of the plant to form salts. They are chiefly acid salts and at once impart an acid reaction to water in which the pea-meal is steeped. If this is left standing at room temperature chemical changes begin at once under the influence of an enzyme (phytase), and increasing quantities of inorganic phosphates are formed. Heat and the chemical processes of extraction induce a more rapid breakdown of the original organic phosphorus compounds, and according to R. J. Anderson[1] a number of lower esters of inosite (mono-, di- and tri-) form, all of which readily undergo conversion to inorganic phosphates.

Phytic acid, although known previously, was first more particularly examined by Posternak[2] who isolated from different cereal and leguminous seeds a white powder, 1·5–2·2 per cent. of their weight, soluble in water and described as a mixture of acid CaMg salts of phytic acid to which he gave the name phytin. It is stored in the cells of the aleurone layer and serves as a reserve of phosphorus for the nourishment and development of the embryo. He found it also in the roots, bulbs and tubers of many other plants. It is derived from the soluble inorganic phosphates of the soil which are transformed in the leaves under the influence of sunlight into soluble organic compounds, and these in turn are transported to the seeds, bulbs, etc., for future use. Animals as well as plants are able to synthesise organic from inorganic phosphates and also to break down the former into the latter.

The free acid is syrupy in consistence, colourless, has a very acid reaction and is readily soluble in water and alcohol. Chemically it is inositehexaphosphoric acid and on hydrolysis yields inosite and phosphoric acid, $C_6H_{24}O_{27}P_6 + 3H_2O = C_6H_{12}O_6 + 6H_3PO_4$.

Its constitutional formula is still unsettled and even its empirical formula is in dispute, as Anderson makes it $C_6H_{18}O_{24}P_6$. It behaves as a polybasic acid and with alkalis, alkaline earths, and the heavy metals, forms neutral salts, two series of acid salts, double salts and acid double salts. The alkali salts are very soluble in water and do not crystallise, the salts of the dyad metals are insoluble in water but very soluble in mineral acids, while the Ca, Mg, Ba and Sr salts are insoluble if neutral, but soluble if acid. Solutions of the acid alkali salts, however, hold the insoluble compounds of the alkaline earth metals in solution and by concentration of these the double salts can be obtained crystalline. The NaCa salt for instance has the formula $C_6H_{12}Na_8Ca_2P_6O_{27}.8H_2O$.

The toxicity of phytic acid and its neutral sodium and acid calcium salts has been determined by Gilbert and Lippmann[3] and by Sécheret[4] who found that the lethal dose depended in an unusual degree on the channel of administration. When given intravenously to rabbits and guinea-pigs about 50 mg. per kg. of body weight were rapidly fatal, hypodermically about 3 g. were required, and per os 10 g. although much more was sometimes tolerated. In a dog 5 g. of the acid calcium salt per os had no visible action, but Mendel and Underhill[5] state that 0·58 g. of the free acid given per vein was immediately fatal. Starkenstein[6] swallowed 10 g. of commercial phytin and experienced no effects of any kind.

The general conclusion is that when taken per os the poisonous organic phosphates are resolved by the bacteria of the bowel into the much less toxic inorganic alkaline phosphates, but individual animals of the same species showed considerable diversity in their ability to do this.

When given hypodermically there was often much local irritation followed by an eschar. With a sufficient dose, however administered, the chief symptoms were dyspnoea, clonic-tonic spasms, tetanus and death. General sensibility was maintained. Post-mortem the heart was in diastole and the viscera congested.

[1] *J. Biol. Chem.* 1912–15, **11–13, 17, 18** and **19**.
[2] *C. R. Acad. Sc.* 1903, **137**; *C. R. Soc. Biol.* 1903, **55**, 1190.
[3] *La Presse Méd.* 1904, No. 69.
[4] *Thèse de Paris*, 1904.
[5] *Amer. J. Physiol.* 1906, **17**, 75.
[6] *Biochem. Ztschr.* 1911, **30**, 56.

Commercial phytin in doses of 1–2 g. per day has had extensive trial in the treatment of rickets and neurasthenic conditions and as a general tonic, but has not proved of value therapeutically.

ISOLATION OF ACTIVE SUBSTANCES

The method of extraction to be described results in more or less decomposition of the phytic acid salts originally present into a number of other bodies, three of which were separated pure and in sufficient quantity to study their action on animals. One was a lower phosphoric acid ester of inosite and possibly others may be present. However that may be, all researches on the subject are in agreement that phytin taken per os in vegetable foods or given as a salt is broken up in the bowel into inorganic phosphate and is excreted as such in the faeces or after absorption in the urine, and seeing how poisonous the organic inositephosphoricacid esters are if absorbed into the blood it is highly necessary that they should very readily break up into less noxious bodies when consumed in food.

Ground undecorticated peas were macerated in chloroform water, the liquid expressed, boiled to throw out albumen and the albumen removed by filtration. It was then precipitated with lead acetate, the precipitate filtered off, and the filtrate further precipitated with mercuric acetate.

(1) The lead precipitate after washing was decomposed with H_2S, the lead sulphide filtered off, the filtrate concentrated *in vacuo* and precipitated by strong alcohol. The precipitate can be purified by redissolving in water and reprecipitating with alcohol, but the loss is considerable. It forms a pure white powder, very soluble in water and very acid in reaction, and is an acid CaMg phytate giving dense precipitates with the acetates of lead, copper, and mercury, with ammonia, sodium hydrate and bicarbonate. On dissolving it in water and neutralising with sodium hydrate or bicarbonate a small and varying amount of a white powder is thrown down insoluble in water but freely soluble in mineral acids—a neutral MgCa salt of phytic acid. Neutral sodium phytate is left in solution and can be precipitated by alcohol as a pure white powder. My experiments on animals were made with this sodium salt, as the original acid CaMg salt and the insoluble salt are unsuitable for hypodermic or intravenous administration.

(2) On distilling the alcohol which was used to precipitate the product obtained from the lead precipitate there is left a very acid yellowish liquid containing a large amount of free phosphoric acid and on neutralising this with sodium hydrate, crystals of sodium phosphate ($Na_2H\,PO_4.12H_2O$) separate out and can be obtained quite pure by recrystallisation from water.

(3) The mercuric acetate precipitate was washed, decomposed by H_2S, the filtrate concentrated *in vacuo* and precipitated by absolute alcohol. When purified by repeated solution and reprecipitation it forms a white powder, very soluble in water, acid in reaction, giving a dense precipitate with mercuric acetate but none with the acetates of lead or copper or with ammonia or sodium hydrate or bicarbonate. The ash contained no calcium or magnesium but gave reactions for phosphoric acid, potassium and sodium, and presumably it is a lower phytic acid ester of inosite in combination with these alkalis. The alcohol used to precipitate it left on distillation a small residue containing negligible amounts of organic and inorganic phosphates and impurities.

The yield from the lead and mercury precipitates and of free phosphoric acid is very variable, depending on the amount of chemical decomposition

which happens to take place in the individual batch under extraction. The following figures illustrate this:

Wt. of peas	Lead precip. alc.-insol. part. An acid CaMg salt	Lead precip. alc.-sol. part. As sod. phosphate	Merc. precip. alc.-insol. part. An acid NaK salt
kg.	g.	g.	g.
1	1·60	3·3	3·75
1	2·65	2·75	—
2	1·65	7·3	9·3
12	4·0	—	52·0
½	0·27	1·3	1·65

Owing to the chemical changes which take place during extraction the phytic acid salt precipitated from the watery extract by lead acetate is not always the same or a single substance. The bulk of it is usually an acid CaMg salt, but it may contain other bodies. Sometimes it gives no precipitate on neutralising with an alkali and sometimes it is not completely soluble in water, but the neutral sodium salt can always be obtained from it pure and without difficulty.

Action on animals

(i) *Water extract of the peas*

Monkey. One kg. of ground lathyrus peas was extracted with water, the water concentrated *in vacuo* to 80 c.c., and one half given to a *Rhesus* monkey by stomach tube. It became restless and uneasy but showed no very definite effects, and 2 hours later the other half was given. In 15 min. it had become very apathetic and sat bent up and inert or lay over on its side. If roused it moved about slowly and carefully and was quite unable to jump. The symptoms were general paresis and somnolence and they lasted all day. Next day it was well and active, but hardly normally agile.

It is evident that there is a limit to the extent to which the toxic organic phosphorus compounds present in the peas can be rapidly broken down in the bowel, and when the limit is reached they are absorbed and exert a marked action on the brain and spinal cord. This explains the numerous cases of acute poisoning of farm stock which have occurred after a large feed of lathyrus.

(ii) *Neutral sodium phytate*

This was obtained from the lead precipitate of a water extract as previously described.

Monkeys. A *Rhesus* monkey was given hypodermically 1·4 g. dissolved in water. It was affected in 5 min. and lay on its side or sat huddled up with its back bent and its head down on its abdomen. It was very weak, could not jump, climbed with an effort, shuffled along with its leg joints flexed, and allowed itself to be freely handled. Two days later it seemed quite well and remained so to all appearance for 15 days, when it suddenly had a series of spasmodic nervous attacks lasting half the day. Each began as coarse muscular jerkings rapidly developing into clonic-tonic spasms, during which it lay

helpless on its side for 2 or 3 min. In the intervals between the attacks it ran about and ate fruit but was far from agile. Its legs were slightly weak for more than a month afterwards, but complete recovery took place. I have previously drawn attention to these attacks as occurring in monkeys long after a hypodermic dose had been given, and in horses 8 weeks after lathyrus feeding had been stopped[1].

When given per os 2 and 3 g. had no visible action. There was no diarrhoea.

Rabbits. A large rabbit (2800 g.) showed no symptoms after 0·7 g. hypodermically, but after 1 g. its legs were definitely paretic.

A much smaller rabbit (575 g.) after 1 g. hypodermically became gradually completely paralysed and died in 5 hours. There was considerable local irritation.

A rabbit (1930 g.) died in convulsions during injection into the ear vein of a solution containing 0·25 g. in 1·5 c.c. water, when only about one-third of it had been given.

Frogs. In frogs 0·02–0·05 g. hypodermically caused depression or complete paralysis of the brain and spinal cord for 24 hours or longer followed by increased reflexes lasting several days.

(iii) *The mercury precipitate*

The organic phosphorus compound obtained from the mercuric acetate precipitate is much less toxic than sodium phytate. As it is an acid salt it was administered neutralised with sodium hydrate.

Monkeys. A *Rhesus* monkey (2225 g.) received 3 g. hypodermically. In 5 min. it lay down and during the whole day was paretic but could run about. There were muscular tremors, a shuffling gait, and a marked general lessening of alertness and agility. Next day it was still weak and it took 12 days to recover fully.

Per os 3 g. dissolved in water had no perceptible action of any kind.

Rabbits. A rabbit (1550 g.) after 1 g. hypodermically became in 5 min. weak on its legs and lay down. It was unable to move about for a time, but had recovered in 3 hours.

When given by the ear vein doses up to 4 dg. had no visible action. After 1 g. a rabbit (1900 g.) became in 15 min. depressed and drowsy and in an hour it sat somnolent with small pupils and its chin resting on the table, but it could be easily roused. Next day it was very quiet and depressed and did not eat, but it had recovered on the third day.

Frogs. With 0·05–0·1 g. hypodermically there was marked depression of the brain and spinal cord followed by increased reflexes.

(iv) *Sodium phosphate*

Sodium phosphate obtained by neutralising the phosphoric acid produced by decomposition of the phytic acid in lathyrus peas is identical with Sodii Phosphas B.P. The amount of it which can theoretically be furnished from

[1] *Edin. Med. J.* 1917, Nov.

the organic phosphorus of the peas is very considerable. In one sample of undried peas the P reckoned as P_2O_5 equalled 0·385 g. per cent., and if this were completely converted into sodium phosphate ($Na_2H.PO_4.12H_2O$) as seems to happen in the intestine it represents 19·4 g. per kg. of peas.

Sodium phosphate is used medically as a mild saline purgative in doses up to 16 g. and has apparently no further action, presumably because, like other saline cathartics, it is not absorbed to any great extent.

Binger[1], however, found that given intravenously to dogs it caused tetany and a diminution of the blood calcium. He was of opinion that the tetany is not entirely due to the drop in calcium as this often occurred without tetany. Tindal[2] confirmed these findings and thinks it improbable that the tetany is dependent on a decrease of the blood calcium.

Salvesen, Hastings and McIntosh[3] found, also in dogs, that small doses of alkaline phosphates per os could be tolerated without any visible effects, but that after three or four large doses the animals became depressed, suffered from tetany, rigidity and twitching of muscles, and finally were unable to move and lay down with extension spasm of the legs. They attribute the symptoms to a drop in the blood calcium and state that calcium chloride (0·5–1 g.) given intravenously restored the animals to normal in 15 min.

Jepsson[4] found that 10–20 g. sodium phosphate given per os to children caused typical tetany coming on in ½ to 2 hours and lasting 2–4 hours, sometimes with clonic-tonic seizures and unconsciousness. With dogs and rabbits similar symptoms occurred. He also states that children fed on the whey of cows' milk (which contains 5–8 times more phosphates than human milk) become spasmophilic and that with removal of the phosphates the spasmophilia ceases. His conclusion is that the action cannot be assigned solely to diminution in the blood calcium although this is not without some effect.

Freudenberg and György[5] ascribe phosphate tetany to a lessening of the ionised calcium in the blood serum by the phosphate ions binding the Ca ions and inactivating them.

Monkeys. A *Rhesus* monkey was given hypodermically 1 g. sodium phosphate from lathyrus. In an hour it was weak and depressed, sat bent up and moved heavily and slowly. Next day it was still rather weak.

Another *Rhesus* received 2 g. hypodermically. It lay down a great deal and had coarse spasmodic jerkings of groups of muscles. After an hour 2 g. were again given and in a short time it was lying on its side with all its joints flexed and unable to move. Its brain was quite alert and it drank milk freely. Four days were required for gradual recovery.

This second experiment was repeated exactly with Sodii Phosphas (B.P.) and the same series of symptoms resulted. One of the monkeys got 2 g. calcium chloride in water per os and another 1 g. calcium gluconate subcutaneously without this in any way affecting the course of the poisoning, a result which lends support to the view that the action of these phosphorus compounds is a direct one on the nervous system and not indirect through a drop in the blood calcium. This view is strengthened when one considers the very small amount of neutral sodium phytate which causes violent spasm when injected

[1] *J. Pharm. and Exper. Therap.* 1917, **10**, 105. [2] *J. Biol. Chem.* 1922, **54**, 35.
[3] *J. Biol. Chem.* 1924, **60**, 311. [4] *Zeitschr. f. Kinderheilk.* 1921, **28**, 71.
[5] *Jahrb. f. Kinderheilk.* 1921, **96**, 5.

directly into the blood stream, an amount which cannot seriously disturb the calcium balance of the serum.

Rabbits. A rabbit (1750 g.) after 2 g. in 10 c.c. water hypodermically became weak in the legs and lay flat for 3 hours, after which it rapidly recovered.

A rabbit (1920 g.) was given by the ear vein 1 g. in 3 c.c. water. It became very quiet and somnolent with smaller pupils. Half an hour later 1 g. was again given, when it immediately went into clonic spasms and died. The heart was still beating and there were no visible lesions.

Frogs. After 0·1 g. hypodermically there was paresis, the frog being just able to crawl about, succeeded by a slight increase in the reflexes which continued for a few days. After 0·2 g. a frog became completely paralysed in 5 min. and remained so till next day, when it died. The action is on the brain and spinal cord.

(v) *Sodium inositehexaphosphate*

This is made from a plant extract by the firm Schuchardt (Görlitz) and is stated to contain 18·5 per cent. P. It is a white powder, very soluble in water, very acid in reaction, and gives no precipitate with alkalis. It is an acid salt and for administration was made slightly alkaline with sodium bicarbonate. Given in this form its action is very similar to that of neutral sodium phytate, but whether it is identical is difficult to decide, as individual animals vary a good deal in their reaction to these poisons.

Monkeys. In a *Rhesus* monkey (2731 g.) 2 g. and 4 g. given per os on consecutive days acted merely as saline purgatives. There was no general disturbance and the appetite remained unaffected. The same monkey after 1 g. hypodermically became weak and feeble in its movements and lay down a great deal, but had recovered by the following morning. This injection caused much local irritation and a slough.

Rabbits. A rabbit after 5 g. in 15 c.c. water per os became in half an hour depressed and drowsy for about 3 hours.

A rabbit (1090 g.) with 1 g. hypodermically became in a few minutes drowsy and inert and unable to sit up, and this condition was followed by tremors and clonic spasms. In half an hour it was helpless and died shortly after from paralysis of the brain and spinal cord. The heart was beating after death and there were no apparent lesions.

Per vein 0·2 g. dissolved in water (weight of rabbit 1980 g.) caused at once violent general convulsions and death.

Frogs. Doses of 0·03–0·05 g. hypodermically were quickly followed by paralysis, passing after several hours into increased reflex irritability. Smaller doses caused sluggishness.

(vi) *Inositehexaphosphate* CaMg

This was bought from Schuchardt and is a white powder stated to contain 22 per cent. P. It is a combination of phytic acid with CaMg, soluble in water,

acid in reaction, and on neutralising with sodium hydrate throws down a dense white precipitate of insoluble CaMg phytate, leaving sodium phytate in solution. Its acid reaction and its decomposition by alkalis render it unsuitable for administration except by the stomach, hence only a few experiments were made with it (unneutralised).

Monkeys. Given to a *Rhesus* monkey per os, 2 g. dissolved in water had no action, but 4 g. and 6 g. acted as saline purgatives without further disturbance of any kind.

Rabbits. A rabbit (2470 g.) got 10 g. per os and in 2 hours had spasmodic movements of the head, tremors, and hurried respiration which lasted all day. Next day it seemed quite well.

A rabbit (1100 g.) after 8 g. per os became depressed and paretic and had diarrhoea. During the next 2 days it gradually became more and more paralysed and was found dead on the fourth day. The post-mortem findings were negative.

It is evident that these two rabbits proved unable completely to break down in the intestine the organic phosphorus compound and were poisoned by its absorption. They reproduce exactly the course and termination of acute lathyrus poisoning in cows as described by Vazeux[1].

When 1 g. (unneutralised) dissolved in 6 c.c. water was given subcutaneously to a rabbit, it caused drowsiness and progressive paresis, deepening in 2 hours to complete paralysis from which it did not recover. The injection caused severe local irritation which spread through the abdominal wall to the peritoneum and hastened death.

Frogs. It can be given hypodermically to frogs without severe local irritation. The action is the same as with the sodium salt, but slightly larger doses are required.

(vii) *Phytin*

Phytin is a trade name protected by a German patent. So far as examined its chemical properties and toxic action were similar to those of the preceding substance.

Summary

A watery extract of lathyrus peas and various salts isolated from them exert a marked action on the brain and spinal cord.

The salts are organic and inorganic phosphoric acid compounds, all of them having an essentially similar action, but differing in toxicity and in their local irritating effect.

Moderate doses cause torpor and depression, larger doses clonic-tonic spasms.

The action is probably a direct one and not indirect through causing a drop in the blood calcium.

[1] *J. Méd. Véter.* 1923, 481.

When given intravenously they are extremely poisonous, very much less so hypodermically, and enormously less so by the stomach.

Given by the mouth, the toxic organic phytates are usually converted in the bowel wholly into the much less toxic inorganic phosphates and may do no harm, but if they escape this decomposition they are absorbed and cause acute or chronic poisoning in animals and lathyrism in man.

Feeding with large quantities of lathyrus peas and the administration of phytates hypodermically or by the stomach cause degeneration of the nerve cells and nerve fibres of the cerebro-spinal and sympathetic nervous systems.

(*MS. received for publication* 9. I. 1934.—Ed.)