THE TOXIC PRINCIPLES IN LEGUMINOUS SEEDS AND CEREALS.

A Thesis submitted in accordance with the Regulations of the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science

By

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THE TOXIC PRINCIPLES IN LEGUMINOUS SEEDS AND CEREALS.

There is but little doubt that some of the most important contributions to Medicine in the last few years have come through the study of dietetics and nutrition. Despite these advances in knowledge, which have, or ought to have, affected the health of all mankind, it is obvious that the subject of dietetics is still in its infancy. This is particularly so in regard to our everyday articles of food. The cause of much of the suffering and ill-health experienced in the world to-day, especially amongst poverty-stricken peoples, could be made manifest by a more thorough examination of staple diets. To quote Sir Frederick Gowland Hopkins (1), "To understand completely what are the conditions upon which perfect health depends, and to know with exactitude why this or that factor or circumstance disturbs it, chemical knowledge must be sought. Its possession will strengthen the art of medicine, whether applied to the prevention or the cure of disease."

Recent investigations on the subject of diet have not only brought to light the importance of quality of the food, but have also emphasized the necessity of balance among some of the essential constituents. Indeed the study of dietetics leads one to agree with Jules Bordet when he says, "La vie est le maintien d'un équilibre incessamment menacé."

The relation of diet to disease has a long history, for man has found "death in the pot" throughout the ages. The causes of illness resulting from the ingestion of food may be divided into six groups:

1. (a) Errors in quantity.
   (b) Errors in quality.
2. The presence of chemical poisons of non-bacterial origin.
3. The idiosyncrasy of the individual.
4. Infectious diseases carried by foodstuffs (e.g., milk).
5. Other infections and intoxications of bacterial origin, known as "bacterial food poisoning."
6. The presence of animal parasites.

The present research was undertaken primarily to investigate the presence of chemical poisons in the commoner peas and cereals, in an attempt to explain if possible the aetiology of the diseases, lathyrism, pellagra and beri-beri. It has long been recognized that these important diseases are due to dietaries containing too much lathyrus, rice and maize respectively, but the exact modus operandi of these foods has so far not been satisfactorily explained. It is of the greatest importance for the purpose of placing human nutrition and animal breeding upon a sound scientific basis to have, in addition to a full understanding of the essential factors in the diet for growth and the promotion of well-being during maintenance, a thorough knowledge of the causes which are responsible for the malnutrition of a human being or animal when confined to a single naturally occurring food substance, such as maize.

Pulses of all kinds have long been cultivated by man as food for himself and his domestic animals. They are especially of very great importance in many Eastern countries, since they are used either alone or to supplement rice, millet, wheat and other cereals; hence the Hindu proverb which says, "Rice is good, but pulses are my very life." They constitute a primary necessity in these vegetarian populations, since the fertility of the soil could not easily be maintained without rotations of leguminous crops, a fact which was well known to the ancients (Virgil, "Georgics"). The extent to which they are grown in India is evident from the following table showing the number of acres of rice, wheat and pulses cultivated in 1927-28 in three important provinces.

Relative importance of rice, wheat and pulses.

	Rice.	Wheat.	Pulses.
Punjab	940,625	9,024,595	5.496,993
Bombay	3,151,509	1,875,200	2,549,148
United Provinces	7,298,299	7,531,333	6,989,110

They have been considered almost entirely as sources of vitamins, carbohydrate and protein, and it is on this basis that their dietetic value has been appraised. With perhaps two notable exceptions, the bitter vetch (Ervum ervilia) and the vetchling Lathyrus (L. sativus and L. cicera), which are mentioned by Hippocrates in his "Epidemics," there has been little suspicion on the part of mankind that leguminous seeds might be poisonous.

The Greek and Roman writers on agriculture and natural history refer to, and sometimes lay stress on, the stimulant properties of the pulses both for man and domestic animals. In view, however, of the findings in the present research, it is worthy of note that among ancient nations some of the pulses were reckoned unclean; for example, Pythagoras condemned the bean to his disciples because its constant use dulled men's wits and caused many dreams; others declared that therein dwelt dead men's souls and therefore, at least "the bishop should not eat beans." From a study of the pertinent scientific literature, it appears that the consumption of peas has generally been kept within the limits of safety and in consequence there have been few outbreaks of disease which might have attracted attention.

Cereals have always formed the major part of man's diet and have generally been regarded as perfectly wholesome. Gross over-consumption of maize and rice, however, has given rise to pellagra and beri-beri respectively. A very vast amount of literature dealing with the many problems of pellagra has been amassed and Winkelman (2) in 1926 found 2,000 articles relating to the aetiology, clinical or pathological findings. It is strange that with so much interest displayed, so little progress has been made in knowledge concerning it. The general opinion has been to inculpate the dietary (usually maize), but its exact manner of operation has never been properly understood. Many have suspected, but none has shown the presence of toxic substances in the food. The volume of literature concerning the many problems of beri-beri, especially in its relationship to vitamins, must be overwhelming. Here again, no one has isolated poisonous substances from the grain, although several have suspected their presence. There are also several scattered references in medical literature to the toxic effects of gross over-consumption of wheat, rye (nervous ergotism), millet and oatmeal (rickets), but the precise modus operandi of these foods has never been satisfactorily tested or demonstrated by experiment in the laboratory or clinic.

Notwithstanding these, the general consensus of opinion has always been rightly to regard cereals as perfectly wholesome when they form even a considerable part of a mixed dietary; even Lind in his "Treatise of the Scurvy" says, "It is certain nothing can be more wholesome than the mealy seeds of several plants, as wheat, barley, rice, etc., as also several of the legumina; and for this reason, because an oil seems necessary to the composition of the animal emulsion; and these in particular contain a vegetable one, of mild and friendly qualities to the human body. They afford so wholesome a nourishment, that they are used by the generality of mankind for the greatest part of their food."

It will be evident that the present research opened out a completely new field of investigation, and one which is important from the point of view of feeding the human population and farm stock of the world. As often happens in pioneer work of this kind, many of the early attempts to isolate active principles from the grains and pulses failed, but with further experience more success was attained. Owing to the large amount of work and time involved in the preparation of the physiologically active products, the writer directed his efforts rather towards the demonstration of the inherent toxicity of the commoner pulses and cereals, than to the isolation of the pure active principles, although in several cases attempts, which were only partially successful, were made in this direction. This problem of isolation of pure chemical substances will require further investigation.

The presence of poisonous substances was demonstrated in the commoner leguminous seeds and also in the cereals.

PART I.

LEGUMINOUS SEEDS.

Leguminous Seeds.

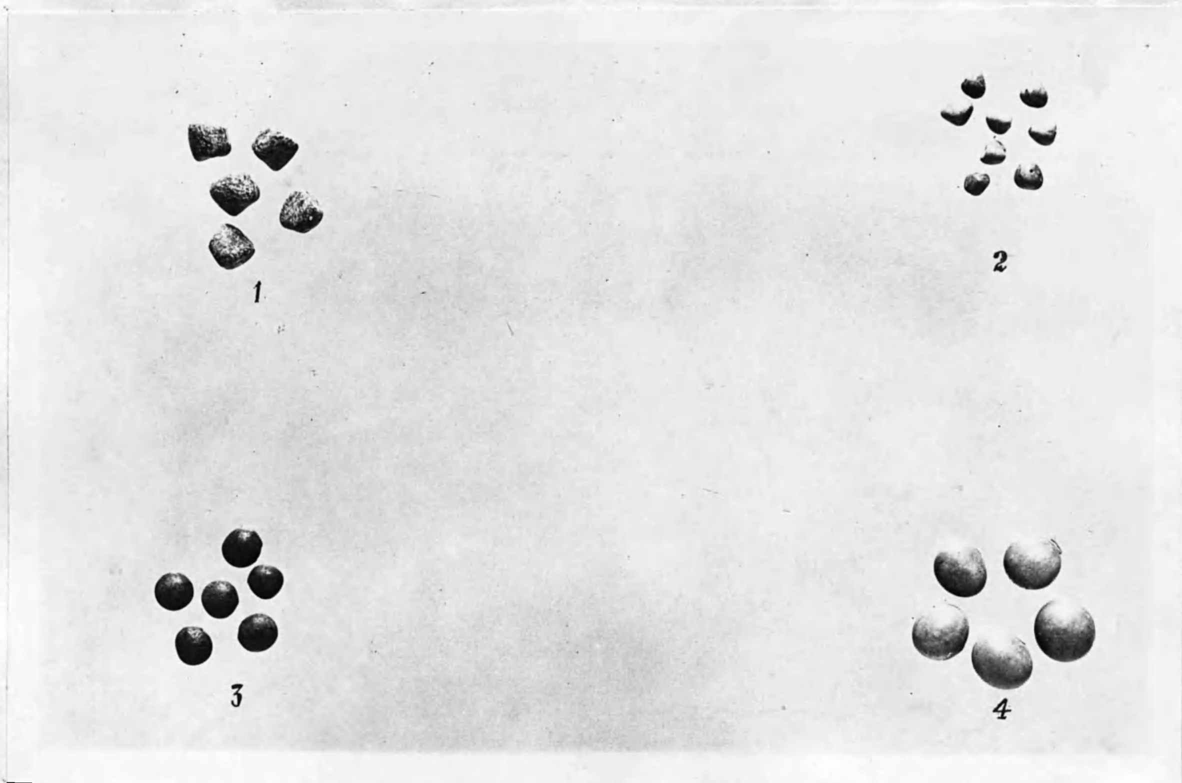


PLATE 1.

Fig. 1. Lathyrus peas (L. sativus);

Fig. 2. Bitter vetch peas (Ervum ervilia);

Fig. 3. Lentils (Ervum lens);

Fig. 4. Soya beans (Glycine Soja).

Leguminous Seeds.

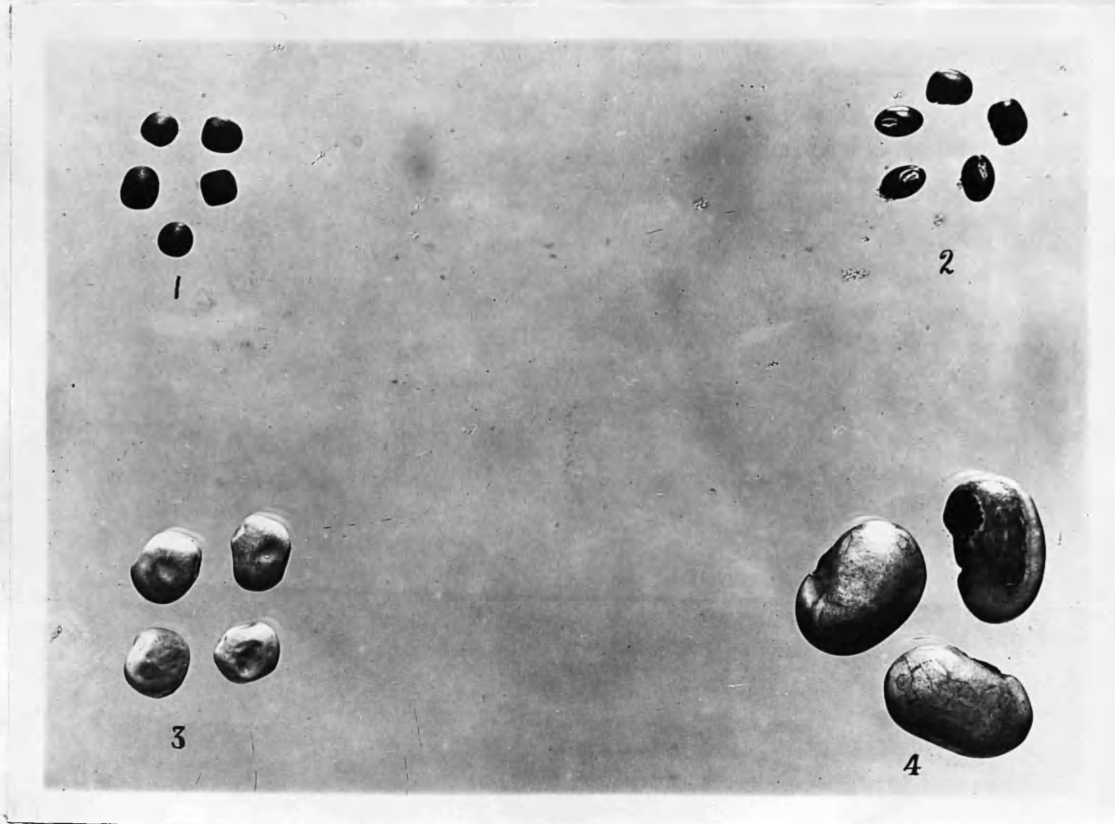


PLATE 2.

Fig. 1. Russian tares (Vicia sativa);

Fig. 2. Pigeon peas (Cajanus indicus);

Fig. 3. Green peas (Pisum sativum);

Fig. 4. Haricot beans (Phaseolus vulgaris).

LEGUMINOUS SEEDS.

General.

The natural order Leguminosae is the second largest in the plant kingdom and includes 600 genera and approximately 12,000 species. These have very diverse properties, some being nutritious, others purgative and astringent and still others poisonous. From a toxicological point of view, however, it may be said that the order, much as its fruits and seeds are used, is in some respects to be regarded with suspicion.

The seeds, generally known as pulse, differ chemically from the cereal grains in several particulars. They sometimes contain rather more oil or fat, a constituent which may rise even to 18 per cent. (in soya beans) or to 50 per cent. (in pea-nuts). They rarely yield less than $2\frac{1}{2}$ and often as much as 4 per cent. of mineral matter or ash. Although the percentage of protein matter in pulses may vary from 18 in the Inga bean to 35.3 in the soya bean, it is always greater than in cereals. This protein matter, which is characteristic of the pulses, is often called legumin, but in reality, it varies slightly in different kinds of pulse, and is not a single definite compound, but a mixture.

The following table gives the mean figures for the composition of the pulses, (from the analysis of the twenty pulses mentioned by Church in his "Food Grains of India").

Composition of the Pulses.

	Mean.	Limiting Figures.
Water	11.7 per cent.	7.5 - 14.6 per cent.
Proteins	25.1 "	7.1 - 35.3 "
Carbohydrates	53.0 "	11.7 - 67.9 "
Oil	2.1 "	.6 - 50 "
Fibre	5.0 "	1.0 - 13.5 "
Ash	3.2 "	1.8 - 4.6 "

Owing to their large content of oil, leguminous seeds are generally regarded as more nutritious than the cereals, although the digestibility of their proteins, compared with that of the corresponding compounds in the cereal grains, has been usually regarded as low. On account of the large quantity of gas generated in their digestion, they have been at all times noted for occasioning flatulence, and sometimes colic pains.

Lathyrus Peas (L. sativus and L. cicera).

The family Papilionaceae, to which lathyrus belongs, includes such well known plants as the lupine, whin, broom, clover, medicago, vetch, scarlet runner, pea and lentil. Its sub-family Viciae contains a very large number of species, several of which are indigenous to Britain and have been fairly extensively grown as forage for horses and cattle without arousing any suspicions that they might be poisonous; among these are L. sylvestris (the narrow-leaved everlasting pea), L. tuberosus (the tuberous vetchling), L. pratensis (the meadow vetchling), L. hirsutus (the hairy vetchling) and L. aphaca (the yellow vetchling). The two species with which the writer is specially concerned are (1) L. sativus (the chickling vetch) and (2) L. cicera (the flat-podded vetch).

(1) L. sativus — cultivated lathyrus — was originally indigenous in that area which extends from the Caspian Sea to the North of India and spread thence to Southern Europe where it has long been cultivated chiefly as fodder for farm stock, and also to some extent, for human consumption. It is also extensively grown in India as a cold weather crop and has the reputation of germinating on land too dry for other rabí. Statistics of actual cultivation are not available for the whole of India, but the candidate finds that in 1924-5 the Central Provinces devoted an area of 360,000 acres to the crop. The plant, which may grow to

a height of three feet or more, obtains support by its tendrils. The fruit is a small, smooth-winged, several seeded pod. The seeds (Plate 1, Fig. 1.) vary in colour and size but all have the characteristic wedged, angled or hatchet-shaped appearance.

(2) L. cicera is a native of South Europe and Northern Africa and is grown chiefly in France, Italy and Algeria as a fodder plant as well as for human consumption. The brown smooth seeds of L. cicera are very like those of L. sativus and can be very easily mistaken for those by a casual observer.

The seeds of both species, either whole or ground, are cooked in various ways, and eaten like other pulses, or the meal may be mixed with wheat or barley flour and made into bread. In India they form the staple diet of large sections of the poorer classes, since they are cheap, palatable and highly nutritious, and in times of famine the increased consumption has been the cause of many recorded epidemics. In this connection, and apart from any inherent toxicity in the lathyrus peas, the writer would like to point out that scarcely any article of food is complete and properly adjusted, for a staple diet, from a chemical point of view, much less from a combined chemical and physiological aspect. Even human milk, as a food for adults, is far from perfect. From a study of the nutrient-ratio (proportion of albuminoids to starch) of lathyrus, it becomes obvious that the defect is precisely of that kind which can be neutralised by the due admixture of cereals. This to a certain extent accounts for the innocuous nature of the peas when taken as a small part of a mixed diet. The candidate finds that the various writers differ in regard to what constitutes a safe proportion. The Kabyles think that one part of lathyrus flour to five of other flour in bread-making is harmless, while Vilmorin (3) says that one third is dangerous with certainty. Buchanan (4) states that in certain Indian jails, four to six ounces daily per person were given for years without any bad results being observed. Probably the explanation of the non-agreement of the different writers lies in the fact that the peas differ greatly in their toxicity from one district to another, owing to the influences of climate and soil. It would thus seem that the effect of climatic disturbances in modifying the quantity and quality of the crop has not received the degree of consideration which it demands. This difference in toxicity also accounts for the negative results obtained by some investigators in attempting to produce the symptoms of lathyrus poisoning in animals. Watt (5), in his valuable article, points out also that there is a "certain capriciousness" in the effects of the poison on different individuals; certain families and certain members of a family being more or less affected by the poison, while others escape. Men are much more liable to be affected than women, in the ratio of ten to one, and boys more than girls.

Despite much evidence to the contrary, there can be little doubt that lathyrus peas, when eaten in excessive amounts over long periods, are capable of causing poisoning and also of producing pathological changes in the central nervous system and the disease lathyrism in man.

Lathyrism.

The name Lathyrism (suggested derivation, "la," augmentative and "thouros," exciting, impetuous) was first used by the Italian, Cantani (6) to describe the chronic nervous disease in man due to the habitual use as food of lathyrus peas (especially L. sativus, L. cicera and L. clymenum). The disease occurs endemically and epidemically and is presumably first described in the writings of Hippocrates (7), who records that "at Ainos, all, men and women who continuously ate peas, became impotent in the legs." Since the time of Hippocrates much information has been collected regarding the poisonous effects, in man and animals, of these peas especially when they form the greater part of the daily dietary. The seeds of the bitter-vetch (Ervum ervilia) have always been known to be poisoneus, and some writers have attributed the epidemic of paralysis at Ainos to the consumption of this particular pulse. In

1671 the Duke of Wurtemberg issued an edict forbidding the use of bread made from vetch-seeds, as it had been noticed that those who ate such bread suffered from a peculiar stiffness of their legs, although they seldom died. In 1691 Ramazzini (8) reported an epidemic in the Grand Duchy of Modena and says, that like the one referred to by Hippocrates, it arose from feeding on pulses, especially Ervum. Numerous outbreaks of the disease have occurred on a large scale, in Italy, France and Algeria and in addition, many isolated outbreaks on a much smaller scale have been described by different writers. In 1833 the disease was first recognized on a large scale in India in the Sangor territories, where, on account of three successive famines in 1829-31, the people were compelled to eat the vetchlings called khesari, matra or teora. Since then numerous outbreaks have taken place in Sind, Nagpur, the Central Provinces and in the Himalaya districts. One of the largest of these is described by Buchanan (4) in a report on an outbreak of lathyrism in the Central Provinces in 1869-1902, when about 7,600 persons were affected. Irving (9) records that in one district in the North West Provinces of India, as many as 6 per cent. and in another 3.19 per cent. of the inhabitants were affected. The disease is still very prevalent in the central and north-western parts of India.

Symptoms.

One of the first symptoms to arise is pain in the back and weakness in the legs, which increase until symptoms of spastic paraplegia appear. The patient now complains of girdle sensations and walks with difficulty. The gait is characteristic, for the feet are turned slightly inwards and are dragged, or raised with difficulty from the ground. The joints appear so weak that it is difficult to proceed any distance without falling, while the body has a peculiar up and down motion. There is no ataxy and no vasomotor phenomena, but the legs waste very much. The arms are not, as a rule, involved, though the hands may tremble. The superficial and deep reflexes are increased, and ankle clonus is present. The excitability of the affected muscles is diminished. Incontinence of urine and impotence are early and common symptoms. The mind is unaffected.

The disease does not itself end fatally, but a definite improvement is seldom seen except in incipient cases.

Symptoms in Animals.

Many of the symptoms of lathyrism in man can be reproduced in monkeys by feeding experiments. The chief action observed here is a paresis of motor nerves which soon passes off if the peas be discontinued, but may become permanent after prolonged feeding and may ultimately end in death, due to paralysis of the respiratory muscles. Other animals differ greatly in their susceptibility to lathyrus poisoning. Horses seem to be peculiarly susceptible. One case is recorded where one hundred and twenty three out of eight hundred horses became ill, and many died, owing to being fed on peas of Lathyrus sativus. The chief symptoms observed were weakness and paralysis of the legs. The poison appears to be cumulative in its action on horses as in man.

Herbivora (including rabbits, sheep, oxen and buffaloes) and pigs are more or less insusceptible to chronic lathyrus poisoning even when fed entirely on the peas for long periods, although they are apt to develop paralysis of the hind legs or at least weakness and tremors. Nevertheless these animals are susceptible to acute poisoning and cases are on record where pigs, sheep and cattle turned into fields of lathyrus to feed, have died overnight. Ducks, geese and peacocks are readily poisoned by the peas, but pigeons, hens and partridges seem to thrive on them, although they may show slight weakness of the legs. Guinea pigs seem to be peculiarly susceptible to lathyrus poisoning.

The Active Principle.

Historical.

Many attempts have been made to isolate an active principle from lathyrus peas. Teilleux (10) obtained from the seeds of L. cicera a resinous substance, which in doses of a few grammes caused paralysis, twitchings and slow death in rabbits. Bourlier (11) succeeded in poisoning frogs, tortoises and sparrows, with ether and alcohol extracts of the peas. Owing to the motor paralysis followed by death in several hours, he is of the opinion that the poison acts on the posterior and lateral columns of the spinal cord.
Marie (12) obtained from L. cicera peas a small quantity of an alkaloid which, however, caused no characteristic symptoms of lathyrism in man. Asher (13) isolated from these peas a volatile alkaloid, which he called lathyrin, which was doughy in consistence, alkaline, insoluble in water, soluble in chloroform, and which on evaporation formed needles; but he did not perform any experiments with the substance, the action of which remained therefore unknown. Astier (14) isolated a volatile alkaloid which he also called lathyrin, but with which he made no animal experiments. He made some experiments with an alcohol extract, two to eight decigrammes of which, given subcutaneously, caused tremors and finally weakness or paralysis of the hind legs in dogs. Blaise (15) made experiments with a glycerine extract, but the results were unsatisfactory. Smith (16) gave the poisonous substance as prussic

acid, apparently indicating a cyano-genetic glucoside. This, however, can hardly be so, as the poison is cumulative, and may not show its effects for weeks or months, or in man, even years, according to the quantity of peas eaten. In 1917 Professor Stockman (17a) succeeded in isolating an active substance which gave the chemical reactions of an alkaloid, and which on hypodermic injection proved to be poisonous to frogs, mice, rabbits and monkeys. Dilling (18) isolated two alkaloids in minute quantity, which in frogs caused increased spinal reflexes, followed by paralysis, and in mice, weakness and paresis of the hind limbs. Acton and Chopra (19) obtained from germinated and ungerminated peas, the hydrochloride of an active amine, which caused in comparatively large doses, weakness and paralysis of the hind legs in monkeys, guinea-pigs, rats and mice. Anderson, Howard and Simonsen (20) failed to find any alkaloidal poison in a pure culture of L. sativus seeds, and ascribed the poisonous effects of the latter to contamination with the seeds of Vicia angustifolia. Recently, the subject has been approached from another point of view, namely, investigations to ascertain if the pulse possesses a poisonous fungoid parasite that could account for the toxic action. However, Professor Percival failed to discover any such fungus.

The foregoing shows the uncertainty which existed in regard to the nature of the active substance of lathyrus, when the candidate first undertook the present research work.

The investigations have shown:-

(1) That a lathyrus diet can cause in monkeys the characteristic nervous symptoms of lathyrism, death, and also pathological changes in the nervous system identical with those found in horses fed on lathyrus and in human lathyrism.

(2) That lathyrus contains citric acid and also a complex acid substance which when administered hypodermically as a sodium salt causes acutely the same symptoms and pathological changes as a lathyrus diet.

(3) That this acid substance is poisonous to other animals.

(4) That other leguminous seeds also contain the same or similarly acting acids and have actions on animals similar to those of lathyrus.

EXPERIMENTAL.

(1) Preliminary Feeding Experiments.

Monkeys were fed on a diet of lathyrus peas (L. sativus) cooked by steaming, with the addition of milk, or bran and yeast, with fruit daily, to preclude any chance of deficiency symptoms arising. Although the animals varied greatly in their susceptibility to the poisonous action of lathyrus, they all tended to reproduce, more or less, the salient features of the disease lathyrism, as it is seen in man. The following is a typical case.

A small Bonnet monkey (Macacus sinicus), was fed on lathyrus peas (steamed), with the addition of a little bran and yeast and with a plentiful supply of fruit daily. In eight days, the left arm was held up, flexed at the elbow and wrist, and the right arm was also slightly affected. From this time, there was quite definite muscular weakness and loss of activity. On the 25th day, it was more affected, with the left upper arm adducted and the fingers and elbow tightly flexed; the legs were weak, and it sat with the joints very much flexed. For the next four months there was little change; sometimes the paresis improved, and sometimes it became worse. On the 146th day, it suddenly became paralysed and lay on its side unable to move, with its fingers, wrists, elbow, toe, ankle and hip joints fully flexed. On the 189th day, it experienced a very severe spasmodic attack, followed by increased paresis for a few days. The lathyrus feeding was then stopped; the animal was put on an ordinary diet, and in a few days, it had recovered most of its normal activity.

After death, this monkey showed pathological changes in the nervous system, consisting of widely spread degeneration of nerve fibres, and injury or destruction of nerve cells in both the cerebrospinal and sympathetic systems. Similar pathological changes have been noticed in horses which have died as a result of continued feeding on lathyrus and in human lathyrism. These degenerative changes of the spinal cord, etc., are also ~~characteristic~~ of pellagra, convulsive ergotism and pernicious anaemia.

Rabbits (both young and fully grown) proved to be peculiarly insusceptible to lathyrus poisoning. They throve, increased in weight, and grew normally when fed for five months on the peas, cooked by steaming.

Guinea-pigs, on the other hand, when fed on lathyrus meal (steamed), emaciated rapidly, developed slight tremors, spasticity, and weakness in the legs and ultimately died in 8 to 35 days. The addition of calcium lactate, sodium bicarbonate or cod-liver oil to lathyrus diets failed to prevent loss of weight in guinea-pigs.

(2) Chemical and Biological.

In searching for the active principle or principles of lathyrus and other leguminous seeds, the writer has directed his efforts along the lines indicated by the observations of earlier writers. In 1894, McDougall (21) stated that boiling the seeds in water before use rendered them innocuous, since the toxic principle passed into the water. Acton (22) showed that steeping the peas in water before use allowed ducks to thrive on them, where formerly they died, the inference being that the poisonous substance had been extracted by the water which was rejected. Other writers also observed that the bitter principles of the lentil, pigeon pea and bitter vetch could be removed by washing them in ordinary cold water or in a dilute solution of washing soda. Since these facts seemed to show the water-soluble nature of the poisonous substances, the writer has concentrated his efforts towards a very thorough examination of the aqueous extracts of the peas.

In the present investigation both the L. sativus peas grown in India and L. cicera peas grown in France were used, and no differences were noticed in their chemistry.

In the early part of the research work much time and energy were expended in searching for a poisonous alkaloid, glucoside or saponin but without success. Two principles of acidic nature, which proved to be poisonous on hypodermic injection, were finally isolated. One of these was found to be citric acid and the other a much more complex nitrogenous substance of unknown constitution.

The method, by which the poisonous acid substances were prepared, was first perfected for lathyrus peas but was later applied to the other leguminous seeds and the cereals. The stages by which it evolved are as follows.

In the first stage the ground peas were macerated in cold chloroform water for some days, the water expressed and the marc again macerated and expressed. The aqueous extract was then filtered and boiled to precipitate proteins which were removed by filtration. The filtrate was very acid and was further purified by precipitation with mercuric chloride, which removed as an insoluble complex substance any protein matter not coagulated by heat. This precipitate was filtered off and the filtrate was freed from mercury by sulphuretted hydrogen. After passing a current of air through the liquid, it was concentrated at a low temperature to the consistence of a thin syrup. On slowly adding this to a large volume of absolute alcohol, an abundant white flocculent precipitate was obtained, which constituted a highly purified extract of the peas, containing the poisonous nitrogenous substance contaminated with sugars and salts. Owing to its hygroscopic nature, it was necessary to dry the precipitate in vacuo over calcium chloride. The yield proved to be about 4 per cent., but this could be increased by distilling off the alcohol to a small bulk, and again precipitating with absolute alcohol.

In order to allow nothing to pass unexamined, the precipitate obtained with mercuric chloride, after a thorough washing with water, was decomposed by sulphuretted hydrogen and freed from mercury. On concentrating the filtrate from the mercury sulphide precipitate, and on adding it to a large excess of absolute alcohol, an insoluble product (mostly proteins) was obtained which proved to be innocuous when given hypodermically to animals. The alcohol used in the precipitation was also found to contain nothing deleterious.

The following table shows the scheme adopted in the preparation of the physiologically active extract:-

Ground Lathyrus Peas

- Macerated and expressed
  - Aqueous Extract
    - Boiled and filtered
      - Precipitate (proteins)
      - Filtrate
        - Precipitated with mercuric chloride solution and filtered.
          - Precipitate
            - Washed, decomposed with H_2S and filtered.
              - Precipitate (mercury sulphide).
              - Filtrate
                - Concentrated to syrup, added to strong alcohol and filtered.
                  - Precipitate of Proteins. (<u>Inactive</u>).
                  - Filtrate (<u>Inactive</u>).
          - Filtrate
            - Decomposed with H_2S and filtered.
              - Precipitate (mercury sulphide)
              - Filtrate
                - Concentrated to syrup, added to strong alcohol and filtered.
                  - <u>Precipitate of Physiologically Active Extract.</u> (Dried in vac.)
                  - Filtrate (Recovered).

Biological Experiments with Purified Extract.

In 1931 Professor Stockman (17b) published the results of numerous biological experiments which were conducted on monkeys, rabbits and frogs with the purified extract.

Monkeys.

When 3 g. of the dry purified extract was given hypodermically to a Bonnet monkey it immediately became weak and paretic. It lay down on its side, and all its joints were flexed as if the weakened extensor muscles had been overcome by the flexors (Plate 3). At times, it developed irregular tonic and clonic spasms of groups of muscles in which the joints were more firmly flexed. The effects lingered in a modified degree for 4 days.

Another monkey received 3 g. of the purified extract hypodermically and suffered from great weakness. It sat with its back bent and its head down between its legs and looked very drowsy. This effect lasted for several days.

Rabbits.

Two rabbits received each 2 g. of the purified extract hypodermically but showed little or no effects. The hind legs were slightly weak but the effect soon passed off. The insusceptibility of rabbits to lathyrus poisoning was borne out by feeding experiments.

Frogs.

In frogs, .15 to .2 g. of the extract given hypodermically caused depression or paralysis of the brain and spinal cord, with fibrillary muscular twitchings lasting for several hours. This was succeeded by increased spinal reflexes usually lasting for several days. Larger doses were lethal, death usually occurring in a few hours from stoppage of the heart.

Lathyrus.

PLATE 3.

Monkey after hypodermic injection of 3 g. of purified extract.

The next stage in the process involved the further purification of the physiologically active extract in an attempt to isolate the pure active principle.

Experimentally, the filtrate from the precipitate with mercuric chloride (containing the physiologically active principle) was carefully precipitated with a strong solution of lead acetate.

The filtrate from the lead acetate precipitate was first examined. It was freed from mercury and lead by sulphuretted hydrogen and concentrated to a thin syrup. On adding this to a large volume of strong alcohol, a mixture of insoluble, white, hygroscopic substances was obtained, and was dried in vacuo over calcium chloride. This was called the "glucoside mixture" since it was likely to contain any poisonous glucosides present in the peas. Doses of .1 to .2 g. of this mixture when neutralised with sodium bicarbonate and given hypodermically to frogs, proved to be very toxic, producing paralysis and tetanus followed by increase of spinal reflexes lasting for several days. However it was early recognized that repeated treatment of the "glucoside mixture" with lead or mercuric acetate tended to render it innocuous, until finally doses of .2 g. could be given hypodermically to frogs without producing the slightest symptoms. On hydrolyzing the "glucoside mixture" by boiling with a few drops of dilute sulphuric acid, and on setting aside for a few days, long needle-shaped crystals were obtained which proved to be active in small doses when given hypodermically to frogs. Chemical examination, however, showed these to consist of potassium sulphate; the toxic action was due to the poisonous potassium ion.

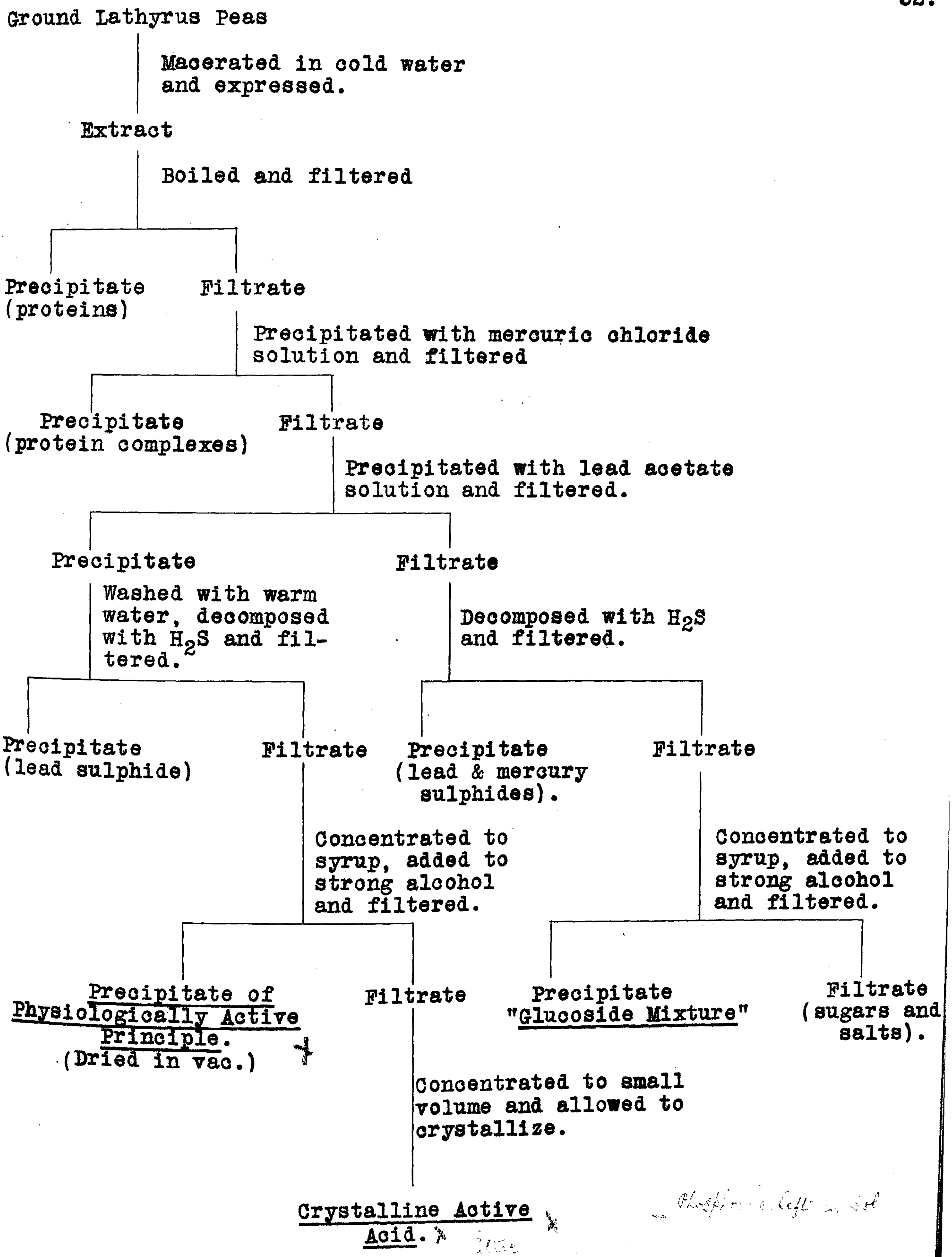
It was thus shown by biological tests that the "glucoside mixture" did not contain an active glucoside while chemical tests showed it to consist of a mixture of sugars and inorganic salts.

The alcohol used to precipitate the "glucoside mixture" was concentrated to a small volume, and on being set aside for some days deposited cubical and needle-shaped crystals. Chemical examination showed the presence of sugars and inorganic salts, while biological tests indicated the absence of any toxic principle.

The lead acetate precipitate was next examined. It was washed with warm water, freed from lead by sulphuretted hydrogen and the filtrate from the lead sulphide precipitate concentrated to a thin syrup. On adding this to a large volume of strong alcohol a white flocculent precipitate of the physiologically active principle was obtained. This was filtered and dried in vacuo over calcium chloride. The yield of this acid substance was usually only .1 per cent, a fact which made its preparation in any quantity exceedingly difficult. It was found to be very toxic when given hypodermically as a neutral body to monkeys and frogs.

The alcohol used to precipitate the physiologically active principle was concentrated to a small volume and on being set aside for some days, usually deposited small rosettes of acicular acid crystals which were dried on a porous plate. The yield (.4 per cent) was small, but probably only represented a fraction of the total amount present in the peas. Later, much larger yields were obtained by using acetone instead of strong alcohol in the precipitation of the physiologically active principle. They proved to be toxic when neutralized with sodium bicarbonate and given hypodermically to frogs, guinea-pigs, rabbits and monkeys, producing symptoms of acute poisoning.

The following table gives the scheme adopted in the isolation of these two active principles:-


Ground Lathyrus Peas
Macerated in cold water and expressed.
Extract
Boiled and filtered
Precipitate (proteins)
Filtrate
Precipitated with mercuric chloride solution and filtered
Precipitate (protein complexes)
Filtrate
Precipitated with lead acetate solution and filtered.
Precipitate
Washed with warm water, decomposed with H_2S and filtered.
Precipitate (lead sulphide)
Filtrate
Concentrated to syrup, added to strong alcohol and filtered.
Precipitate of Physiologically Active Principle. (Dried in vac.)
Filtrate
Concentrated to small volume and allowed to crystallize.
Crystalline Active Acid.
Filtrate
Decomposed with H_2S and filtered.
Precipitate (lead & mercury sulphides).
Filtrate
Concentrated to syrup, added to strong alcohol and filtered.
Precipitate "Glucoside Mixture"
Filtrate (sugars and salts).

Tests with Physiologically Active Principle (Alcohol Insoluble).

Chemical.

The crude active principle proved to be a white, amorphous, hygroscopic substance which was very soluble in water and very acid. Numerous attempts were made to purify it further, by crystallization from water and dilute alcohol, but these resulted only in the formation of a glassy mass which could be easily broken up into flakes. It was insoluble in both cold and boiling ether, chloroform, acetone, benzene and trichlor-ethylene. Its solution in water gave the following reactions:-

(1) Lead acetate gave a dense white precipitate insoluble in boiling water and dilute acetic acid; soluble in strong mineral acids.

(2) Lead subacetate gave a similar dense white precipitate.

(3) Mercuric chloride gave no precipitate even on boiling.

(4) Mercuric acetate gave a dense white precipitate insoluble in boiling water and dilute acetic acid; soluble in strong mineral acids.

(5) Phosphomolybdic acid gave little or no precipitate.

(6) Phosphotungstic acid gave no precipitate.

(7) Tannic acid gave no precipitate.

(8) Picric acid gave no precipitate.

(9) Maeyer's reagent gave no precipitate.

(10) Bouchardat's reagent gave no precipitate.

(11) Potassium hydroxide gave a flocculent white precipitate at the neutralisation point.

(12) Sodium hydroxide gave a flocculent white precipitate.

(13) Ammonium hydroxide gave a white precipitate at the neutral point.

(14) Sodium carbonate gave a faint precipitate in the cold and a larger precipitate on boiling.

(15) Sodium bicarbonate gave a dense white precipitate at the neutralisation point. A larger precipitate was obtained on boiling or on allowing to stand for some days.

(16) Calcium chloride gave a dense white precipitate in the cold but a larger precipitate on boiling.

(17) Magnesium sulphate (saturated solution) gave no precipitate even on warming.

(18) Sodium chloride (10 per cent. solution) gave no precipitate.

(19) Ammonium sulphate (saturated solution) gave no precipitate.

(20) Ferric chloride gave no precipitate but on boiling the colour of the clear solution deepened to a brownish-red tint.

(21) Fehling's solution gave no reduction even after hydrolysis of the acid substance with dilute mineral acids.

(22) Silver nitrate yielded only a trace of a greyish amorphous precipitate soluble in dilute ammonium hydroxide.

(23) Ammonium oxalate gave a fairly dense white precipitate insoluble in dilute ammonium hydroxide; soluble in dilute nitric acid. *

The following tests for proteins and amino acids were applied but with negative results.

PROTEIN TESTS.

1. Biuret reaction.
2. Xanthoproteic reaction.
3. Voisenet's reaction.
4. α-Naphthol reaction.

AMINO ACID TESTS.

1. Ninhydrin reaction.
2. Werner's reaction.

* Purification of the active principle by precipitation of the insoluble lead or mercury salt, followed by decomposition with sulphuretted hydrogen always resulted in a very big loss (80 per cent.) of material, due to absorption on the sulphide precipitate. It was finally further purified by

precipitation with sodium bicarbonate which "salted out" an organic complex of calcium and magnesium, which was filtered off and which, on being tested on frogs, was found to be inactive. On adding the filtrate to a large volume of strong alcohol, a white amorphous precipitate of the sodium salt of the active principle was obtained. This was filtered off and dried in vacuo. The free acid was obtained from it by decomposing the lead salt with sulphuretted hydrogen, and precipitating the active principle with strong alcohol. It was very acid and gave the same reactions as the crude product, but did not give any precipitate with alkalies; attempts to crystallize it from water or dilute alcohol failed. It did not form a derivative with para-bromophenacyl bromide or para-nitro-phenacyl chloride. Many experiments were conducted to obtain a crystalline salt, but these so far have been unsuccessful. The equivalent (by titration against standard barium hydroxide solution) was found to be 145, while a combustion analysis yielded the following results:-

Carbon	=	25.1 per cent.
Hydrogen	=	1.82 " "
Nitrogen	=	1.49 " "

see also p. 99

Biological.

Monkeys.

Professor Stockman (17b) gave hypodermically 0.8 g. of the sodium salt of the active acid to a Bonnet monkey, and in a few minutes it had become very weak and paretic. It could hardly stand and was only able to drag itself about. It lay down much on its side and at times its legs were stretched out as if in a tetanic spasm. This effect lasted all day; on the following day it was merely paretic and on the third day seemed normal.

After death this animal showed degenerative changes in the nerve fibres and chromatolytic changes in the nerve cells of both the spinal cord and brain, similar to those found in the monkey fed on lathyrus peas (and in human lathyrism.)

Frogs.

2 to 3 cg. of the acid (neutralised with sodium bicarbonate) given hypodermically to frogs produced paralysis lasting for several hours, followed by increased spinal reflexes. Smaller doses produced the same symptoms to a lesser degree, while larger doses usually resulted in death.

The substance seems to be a nerve and muscle poison, since the muscles in the neighbourhood af the injection were often irresponsive to the faradic current. Owing to its acidity and calcium-precipitant nature it seems highly probable that there will be disturbance of the calcium and alkali balance in the body.

Tests with Crystalline Acid (Alcohol-soluble)

Chemical.

The acid, which crystallized with difficulty from the alcohol or acetone used in the precipitation of the physiologically active principle, was dried on a porous plate and then re-crystallized several times from water. It formed rosettes of acicular crystals or large rhombic prisms (Plate 4) according to the physical conditions. These were very soluble in water and acetone, fairly soluble in alcohol, but only very sparingly or not at all in ether, benzene, chloroform and tri-chlor-ethylene.

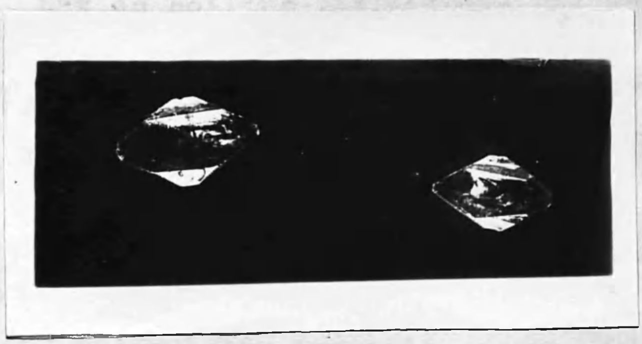


PLATE 4.

Crystals of the acid soluble in alcohol.

They gave the following reactions:-

1. When heated on platinum foil they first melted, gave off water and then decomposed; charring took place and pungent acid fumes were evolved.

2. Lead acetate gave a dense white precipitate, insoluble in dilute acetic acid but soluble in mineral acids.

3. Mercuric acetate gave a white precipitate, soluble in mineral acids.

4. Calcium chloride in a neutral solution produced no immediate precipitate in the cold, but on boiling the solution, a crystalline precipitate was formed. This was soluble in acetic acid but insoluble in caustic potash.

5. Lime water, in excess, produced no precipitate in the cold, but on boiling the mixture for some time a white precipitate was formed which redissolved in caustic potash.

6. Silver nitrate in a neutral solution produced a white precipitate insoluble in dilute nitric acid, but soluble in ammonium hydroxide.

They were finally identified as citric acid by the following results:-

1. The crystals, dried for 48 hours in vacuo over calcium chloride, gave an equivalent of 63.9 when titrated against standardized barium hydroxide solution using phenolphthaleïn as indicator.

2. The acid crystals dried in an electric oven at 60°C. gave a melting point reading of 151°C. A mixed melting point determination with citric acid (dried) gave 150°C.

3. They yielded the following results on combustion analysis:-

Mean results: C = 37.64% ; H = 4.19%.

Citric acid requires: C = 37.5% ; H = 4.17%.

4. Some crystals dissolved in water were precipitated by barium acetate solution and the precipitate digested on the steam bath for one hour. It was filtered off, washed with hot water and dried in a desiccator. The barium content of this salt was then determined, and was found to agree very closely with the result obtained for barium citrate.

Mean result: Ba = 52.3%.

Barium citrate requires: Ba = 52.17%.

5. They formed crystalline derivatives with para-bromo-phenacyl bromide and para-nitro-phenacyl chloride melting at 102°C. and 148°C. respectively. These results are in direct agreement with those obtained by Emmet and Reid (23) in their investigation of the derivatives formed by the interaction of citric acid and the above reagents.

The foregoing data identified the crystals isolated from the alcohol (or acetone) used in the precipitation of the physiologically active principle as citric acid. The following tests were confirmatory:-

1. An aqueous solution of the crystals heated with a dilute solution of mercuric sulphate in sulphuric acid, with the gradual addition of potassium permanganate solution gave a white precipitate. This is a positive reaction of Denigès' (24) test for citric acid.

2. A small quantity of the acid was dissolved in a little water, a few drops of deci-normal potassium permanganate solution added, and the liquid carefully warmed. A few drops of ammonium oxalate were added, then a small volume of sulphuric acid and a few drops of bromine water. The turbidity which appeared showed the presence of citric acid (Stahre's reaction) (25).

3. A small quantity of the acid was dissolved in water and oxidised with 4 per cent. potassium permanganate solution. A small quantity of resorcinol was added and then slowly sulphuric acid till a red colour appeared. After cooling, the mixture was extracted several times with ether. A little water was added to the ether extract and also a few drops of ammonium hydroxide. An intense blue fluorescence, due to the formation of β-hydroxy coumarinacetic acid, showed the presence of citric acid (26).

Micro-test. A micro-drop of concentrated sulphuric acid was added to ·1 mg. of the acid and 2 mg. of vanillin on a watch - glass, and the mixture placed on a steam bath. After 3 minutes it became brownish-violet in colour and after 10 minutes showed a definite violet shade when held against the light. It was now allowed to cool and 3 micro-drops of water added; the colour changed to green. On the addition of 7 micro-drops of ammonium hydroxide the green colour turned to rust red (27).

Although citric acid is one of the common occurring plant acids its presence in lathyrus peas has not previously been demonstrated. Franzen and Helwert (28) mention its occurrençe, however, in the seeds of Vicia sativa, Pisum sativum and Vicia Faba.

Biological.

Several experiments with the sodium salt of the crystalline acid were conducted on monkeys, rabbits, guinea-pigs and frogs by Professor Stockman, the results of which have not yet been published.

Monkeys.

Expt. I. A Rhesus monkey (4 kilos.) was given hypodermically 0.85 g. of the crystalline anhydrous acid neutralised with sodium bicarbonate. In 15 minutes it was evidently weak and lay down on its side from time to time. One hour after administration the weakness had increased; it sat with its back bent and head down, and often leant for support against the wall or lay down on its side with the fingers and toes flexed. One hour later it was much weaker, climbed slowly and carefully, was unable to jump and lay down a great deal. It could still move about on all fours, but had completely lost its agility and alertness. It continued much in this condition for two hours longer when it began to recover and ate freely. Next day it was not nearly so agile as normally and was very easy to handle, but had recovered to a very considerable extent. On the third day it was still somewhat depressed but had apparently suffered no permanent damage to its nervous system and there was no local inflammation from the injections.

When the sodium salt was given per os it was not nearly so poisonous, the inference being that, like many other toxic substances, it can be detoxicated by the body if the dose is not too large and if its absorption is not so rapid as to overtax the detoxicating mechanism.

Expt. II. A Rhesus monkey got per os 2 g. of the sodium salt of the acid dissolved in water. It remained rather quiet, yawned a great deal, and probably had some weakness in the legs. Next day 3.5 g. was given and this was followed by quite definite general muscular weakness, drowsiness and yawning.

On the third day 6 g. was given, after which, during the whole day, it sat huddled up with all its digits flexed, inactive and very weak muscularly. Next morning it was found lying on its back, semi-paralysed and semi-spasmodic with its legs stretched out stiffly and the toes flexed. It recovered sufficiently to be able to hobble about and during the next two days gradually regained its normal activity. On the following morning 1 g. of the sodium salt was given into one of the leg veins, when it died almost immediately.

The heart was found beating when the thorax was opened and continued to fibrillate for ten minutes. The brain, spinal cord, lungs and abdominal viscera were of normal appearance and there was no arterial or venous engorgement. Microscopic examination showed slight cloudy swelling of the convoluted tubules of the kidney. In the posterior root ganglia of the spinal cord and in the sympathetic ganglia (stellate and superior cervical) there were early toxic changes as manifested by swelling of the cells and slight perinuclear chromatolysis. Marchi's staining revealed very early myelin degeneration in the fibres of the sciatic nerve, spinal cord and cauda equina. The brain and cord showed no cell changes.

This is a case of acute poisoning and the pathological findings suggest (1) a very early stage of degeneration of the nervous system resulting from the oral administration of the salt and (2) rapid death due to paralysis of the medullary centres from the intravenous injection.

Rabbits.

Expt. III. In a rabbit (890 g.) 0.75 g. of the crystalline acid (neutralised with sodium bicarbonate) given hypodermically caused almost immediate paresis followed in ten minutes by a series of tonic spasms. Thereafter the animal lay on its side with increased reflexes, dilated pupils, dulled conjunctival sensitiveness, and unable to move voluntarily — a mixture of paresis and hyper-excitability. Occasionally it had a slight tetanic spasm, after which it lay very limp or with slight twitchings of the head and leg muscles. It remained in this condition all day, but next day was sitting up, rather weak in the legs but eating freely, and soon had quite recovered.

Expt. IV. A rabbit (790 g.) got 0.25 g. of the crystalline acid (neutralized) hypodermically and became weak in the legs for about four hours but otherwise was not much affected.

Expt. V. The effect of intravenous administration was tested on three rabbits. When 0.5 g. of the sodium salt, in 2 c.c. of water, was injected into the ear vein death occurred immediately. With 0.25 g. of the sodium salt clonic spasms and collapse came on at once followed after five minutes by tonic spasms which soon passed off leaving a weakness in the legs, but in an hour this also had disappeared. In the third rabbit a similar dose had no visible action.

Guinea-pigs.

Expt. VI. A guinea-pig (517 g.) was given hypodermically 0.5 g. of the crystalline anhydrous acid neutralised with sodium bicarbonate. In 5 minutes it had a series of tetanic attacks and 10 minutes later lay limp on its side as if the spinal cord was depressed, but still having slight abortive spasms — a mixture of paresis and hyper-excitability. Half an hour later it sat up but was very paretic and refused to move about. The hyper-excitability of the cord had passed off. It continued in this state till the late evening, but next day was active, ran about freely and ate well.

Frogs.

Expt. VII. In frogs doses of less than 5 mg. had no apparent action and even with 5 mg. the effect was no more than a very slight sluggishness. With 0.01 g. (neutralised) given hypodermically a small frog rapidly became paralysed and some hours later the heart was found stopped in diastole and all the voluntary muscles inexcitable to the faradic current. With larger frogs and doses of 1 to 3 cg. the initial paralysis gradually lessened to paresis with increased spinal reflexes, often tetanus, passing into recovery or death.

The symptoms produced in the foregoing experiments are identical with those obtained by von Vietinghoff-Scheel (29) and Robertson and Burnett (30) in their investigations of the action of citrates on mice, guinea-pigs and rabbits. It is evident that the action of these substances is essentially acute.

Chronic poisoning in animals, due to the administration of citrates, seems to be unknown or at least, has not been observed. This is probably due to the tolerance acquired to the action of these drugs, since Robertson and Burnett (30) have shown that, "as a result of administering repeated and gradually increasing doses of sodium citrate, rabbits acquire such a pronounced degree of tolerance for the drug that a dose which normally produces extremely severe symptoms of intoxication, or even death, causes either very slight symptoms or else no symptoms of intoxication at all."

Several opinions are held in regard to the precise mode of action of citrates. The general consensus of opinion however, refers their toxic action to their power of diminishing the concentration of calcium ions in the tissues, by converting the calcium into a non-ionic form. The tolerance to sodium citrate may thus be regarded as consisting essentially in tolerance to deprivation of tissue-calcium, and the possibility is indicated that the sensitiveness of tissues to lack of calcium is a function of the 'suddenness' with which calcium is withdrawn, as well as of the absolute amount of which the tissues are deprived. It is possible that the tissues affected by sodium citrate can function at various "calcium levels," provided that the transition from a higher to a lower level is sufficiently gradual. On the other hand, it is possible that the tolerance to deprivation of tissue-calcium, which is brought about by repeated administrations of sodium citrate, is due to the dissolution of reserves of calcium which are ordinarily untouched, but which under the influence of the drug become available to partially replace the calcium, as rapidly as it is withdrawn from the tissues.

The following experiments with citric acid and sodium citrate were conducted on monkeys, rabbits and guinea-pigs, in attempts to produce symptoms of chronic poisoning in these animals. In view of the results obtained however, it is highly improbable that citric acid is the direct cause of lathyrism, although owing to its calcium-precipitant nature it must undoubtedly tend to disturb the calcium and alkali balance of the body, since Caspari (31) has shown that the prolonged administration of at least one calcium-precipitant, to wit oxalic acid, leads to a pronounced diminution in the content of lime in the bones. In this connection it is interesting to record, that a young monkey fed chiefly on lathyrus peas for nearly two years showed very marked bone changes at the time of death. Kobert (32) also notes that the symptoms (neuro-muscular) in chronic cases of oxalic acid poisoning are heightened by the eating of lemons and oranges, owing to their citric acid content.

Monkeys.

A large Bonnet monkey was fed on a diet of bread (cooked by steaming) plus 5 g. of butter, 60 grains of sodium citrate and a plentiful supply of fruit daily. It showed no symptoms after 15 days' feeding and citric acid (60 gr.) was substituted for sodium citrate. Despite the change, the animal was not affected in proportion to what was expected from the large daily dose of acid, and showed either no symptoms, or only very slight weakness in the legs. The experiment was stopped after 83 days' feeding, as the animal seemed to be ~~completely~~ almost unaffected by the acid.

Rabbits.

Two rabbits were fed on a mixture of maize meal (4 parts) and wheat bran (1 part) with the addition of 60 grains of sodium citrate and 60 g. of cabbage daily. They showed no symptoms after 15 days' feeding, and citric acid (60 gr.) was substituted for sodium citrate. After 70 days' feeding their coats were not in good condition but the animals were otherwise normal. Determination of the calcium content in the blood serum of these rabbits after 89 days' feeding gave the following results.

(1) Ca = 11.66 mg. per 100 c.c. of serum.

(2) Ca = 9.54 mg. per 100 c.c. of serum.

Normal rabbit requires Ca = 14 - 18 mg. per 100 c.c. of serum.

One animal died owing to an accident without showing any further symptoms, but the other gradually became weak and was completely paralysed in the hind legs after 127 days' feeding. An X-ray examination of the bones of this animal showed no evidence of rickets (Plate 5), but a determination of the calcium content of the blood serum showed the presence of only 8 mg. of calcium per 100 c.c. of serum. The citric acid was withdrawn from the diet and a salt mixture substituted, when the rabbit recovered and the calcium content of the blood serum became normal. On restoring the acid and withdrawing the salt mixture, it was found that the animal had acquired a complete tolerance to the drug, since it showed no further symptoms even after nine months' feeding.

Guinea-pigs.

Two guinea-pigs were fed on a mixture of maize meal (4 parts) and wheat bran (1 part), plus orange juice and 30 grains of citric acid daily. They lost about one-third of their weight, and died in 20 and 21 days respectively. Their general condition remained good and there seemed no apparent cause of death. This was evidently a case of acute poisoning.

Experiments conducted on frogs by Professor Stockman have shown that the effects (paralysis etc.) produced by the hypodermic injection of 3-5 cg. of sodium citrate can be inhibited by the almost simultaneous injection of a dose of calcium acetate (3 cg.). This is in agreement with the results obtained by Busquet and Pachon (33), who have shown that there is an antagonism between sodium citrate and soluble calcium salts in their action on the heart and on the vagus nerve.

PLATE 5.

Radiograph of hind leg of rabbit receiving 4 g. citric acid daily in food.

(After 127 days' feeding.)

The Phosphate Content.

Lathyrus contains large quantities of inorganic phosphates (chiefly as KH_2PO_4), and these can be recovered from the alcohol-soluble portion of the decomposition product derived from the lead acetate precipitate, by neutralising with sodium hydroxide followed by fractional crystallization, when sodium phosphate separates out in rhombic prisms. In one experiment 75 g. of these were obtained from a 5 kilo. lot of Lathyrus cicera peas.

It is highly probable that the acid phosphate, which is in excess of the calcium content of the peas, plays an important part in the production of lathyrism, since Binger (34), Salvesen, Hastings and McIntosh (35) and Salvesen and Linder (36) have shown that hypocalcaemia and tetany result in dogs after repeated administrations of large amounts of phosphate. The acid salt probably affects the calcium and alkali balance of the body, and tends to lead to increased excretion of inorganic salts, especially calcium. It is quite conceivable that this, in conjunction with other influences, might have a very disturbing effect on the nervous system, since it has been shown by Locke (37) and Overton (38) respectively, that calcium is necessary for the passage of the nerve impulse from nerve to muscle across the myoneural junction, and for the transmission of the excitatory process through the synapses between nerve fibres and nerve cell bodies.

The following biological experiments show the effects of ingestion of repeated doses of phosphate in rabbits and guinea-pigs.

The amounts of phosphate and citric acid in a lathyrus diet were calculated, and were superimposed on a supplemented diet of maize meal and bran. The ultimate effects produced, were due to the total phosphate and citric acid present.

Rabbits.

Two young rabbits (3-5 months) were fed on a mixture of maize meal (4 parts) and wheat bran (1 part), plus 45 g. cabbage, 1.2 g. sodium phosphate and .38 g. citric acid. They gradually lost weight and their coats became very bare. One died after 28 days' feeding having lost nearly one-third of its body weight. Its bones were brittle, thinned and deprived of calcium. The other continued to live, but after 61 days' feeding began to exhibit marked tetanic spasms with violent muscular twitchings. At this stage it was in a miserable condition owing to loss of fur, and the dose of phosphate and citric acid was reduced by half. It continued to show tetanic spasms at intervals, but it increased in weight and the condition of its coat improved. It was still alive after 96 days' feeding.

Guinea-pigs.

Two guinea-pigs were fed on a mixture of maize meal (4 parts) and wheat bran (1 part), plus .38 g. sodium phosphate, .13 g. citric acid and a plentiful supply of orange juice daily. One lost much weight, became weak in the legs and died after 49 days' feeding. A post-mortem examination showed the presence of purulent cysts on the liver and spleen. Although the other maintained its weight and remained in fairly good condition, despite slight weakness of the legs, it died in 92 days. A post-mortem examination showed the viscera to be healthy, except for a 'mottling' on the kidneys suggestive of nephritis.

Conclusion.

Without generalizing further than the observed facts warrant, it is evident that the foregoing experimental work must throw some light on the aetiology of lathyrism. However it must always be understood that studies made upon animals cannot be accepted unreservedly in the domain of human pathology, since they are merely tentative and introductory in nature and must await confirmation by clinical medicine.

The biological work has shown that the disease is not due to lack of vitamins, since all the animals were having vitamin-containing foods in abundance, while the chemical investigation has shown the presence of an acid substance which on hypodermic injection can produce in monkeys, degenerative changes in the brain and spinal cord similar to those found in animals fed on lathyrus and in human lathyrism. The most probable explanation of the action of this substance is that it is not only irritant and poisonous in itself, but that it has the secondary effect of withdrawing inorganic salts, especially calcium, from the blood and tissues. In this connection, the large citric acid and phosphate content of the peas must also play an important part in producing the final complex condition lathyrism.

The Soya Bean (Glycine Soja).

The soya bean (suggested derivation, Japanese shoyu), also known as the soja bean and in the United States as the soy bean, is a medium-sized pulse with a white smooth seed-coat (Plate 1, Fig. 4). It is the seed of Glycine Soja, a small, sub-erect, trifoliate hairy annual with pods generally 3 to 4-seeded. This bean has long formed a considerable article of food in China and Japan, and is mentioned in the "Pen Ts'ao Chin" which was written by the Emperor Shennung in the year 2838 B.C., and which is the first Chinese record that describes the plants of the country. Since 1873 it has been grown as an experiment in some of the warmer parts of Europe, largely through the propagandist efforts of Professor Haberlandt (Vienna), and has been found to grow well in the places where maize also thrives. It is at present extensively cultivated in the warmer regions of India, Japan and China, especially Manchuria where it was first introduced by the Chinese in 1870. The extent to which it is grown in the East can be partly ascertained from the fact that in 1926 Manchuria exported 1,423,000 tons of the beans and 165,000 tons of the oil. From this source Great Britain imported 46,000 tons of beans and 43,000 tons of oil, while Germany consumed 370,000 tons and 20,000 tons respectively.

Small acreages of the bean are also grown in Italy, France, Russia, Hawaii, Egypt, South Africa and South America.

In the Far East, it is of primary importance as an article of food, since it is eaten by all classes and in some form or other at almost every meal. Compared even with other pulses, its chemical composition ~~however,~~ entitles it to a very high place as a food capable of supplementing the deficiencies of rice and other starchy grains. Very few vegetable products are as rich in both albuminoids and fats.

<u>Composition of Soya Bean.</u>

Water	11%
Albuminoids	35.3%
Starch and sugars	26%
Fat	18.9%
Fibre	4.2%
Ash	4.6%

In India it is used to some extent as a dal, boiled like other beans, but in China and Japan three preparations are extensively made from it. Soy sauce is the best known of these, but more important are the soy or bean cheeses (natto, miso or shoyu), and a kind of paste. The sauce is made by mixing the boiled beans with roughly ground wheat or barley, covering for twenty-four hours, and then allowing the mass to ferment. After salting, watering and stirring daily for two months, the liquid is then drawn off, filtered and casked. A special cheese known as Teau-fou is also prepared by the Chinese. The beans are ground with water and the resulting emulsion separated from the cellular tissues by filtration through cloth. The filtrate is brought to the boiling point, cooled, and when almost cold a little salt is added; the coagulated mass, when drained and pressed, constitutes the cheese.

The beans are imported into this country in large quantities chiefly from Manchuria, and after crushing the oil is extracted. It is extensively used for cooking purposes etc., and in the manufacture of soap. The residual meal forms an extremely rich cattle food since it contains 44 per cent. of proteins, 34 per cent. of carbohydrates and 1 per cent. of fat.

On the whole it has proved highly satisfactory as a cattle food, but from time to time cases of poisoning have occurred from its use, sometimes on a considerable scale, and have been recorded in the veterinary journals.

In 1916 Sir Stewart Stockman (39) reported on a fatal outbreak in the South of Scotland, in which 67 cows were affected and of these 54 died. They had all been fed for periods varying from 50 to 71 days, on a mixture of soya meal, hay, bran, crushed oats and fresh grass. Pigs, sheep, very young cattle and those not in milk, although receiving the same feeding, were unaffected. A peculiar feature of the outbreak was the fact that symptoms did not appear for a considerable time, and often only after the use of the soya meal as food had been discontinued. This is very reminiscent of what occurs in lathyrus poisoning, especially in horses. The symptoms were drying up of the milk, bleedings from the nose and bowel, fever and abdominal pain; after death large haemorrhagic lesions were found in the subcutaneous tissues, muscles and viscera. The farmers attributed the illness to poisoning by the soya meal.

Experiments carried out by the Ministry of Agriculture in 1916 and Professor Stockman (17c) in 1927 however, failed to show the presence of any chemical poison in the meal.

From the post-mortem findings it seems likely that the animals died from the effects of scurvy, which had probably been greatly aggravated by the poisonous constituents which have since been shown to exist in the meal.

The present investigation was conducted on the defatted meal and has shown:

1. That a diet of soya meal can produce in a monkey symptoms similar to those found in lathyrus poisoning.
2. That soya contains (1) a physiologically active principle which is similar in its action to

that found in lathyrus and which can be extracted by similar chemical methods and (2) citric acid.

3. That this active principle is poisonous to other animals on hypodermic injection.

Experimental.

(i) Preliminary Feeding Experiments.

Monkeys.

A monkey was fed on soya meal (ad libitum) cooked by steaming, with the addition of fruit daily. After 14 days no toxic symptoms were visible. It was then given in addition drinking water in which 100 g. of the meal had been macerated for 24 hours. The effect of the increase of dose was very marked. After each meal it became inactive and drowsy, and sat very much bent down for 2 or 3 hours. When drinking water from the maceration of 200 g. of meal was given, it became definitely paretic, inactive and drowsy (Plate 6). The experiment was stopped after 31 days, and the monkey gradually recovered when it was put on a normal diet of bread, milk and fruit. After 14 days it was perfectly normal again.

Rabbits.

Rabbits fed on raw soya beans or meal, lost weight steadily and died in 61-93 days. They all developed at times, slight tremors and spasticity in their legs. Rabbits

Soya.

PLATE 6.

Monkey after 31 days' feeding.

on similar diets, supplemented with orange juice, died in a much shorter time (7-17 days) than those fed on soya only. When cabbage was added to a meal diet however, rabbits remained in perfect health, increased in weight and throve normally. The withdrawal of the cabbage resulted in loss of weight and general decline.

Guinea-pigs.

Guinea-pigs fed on soya beans, or meal cooked by steaming, gradually lost weight and died in 18-39 days. Post-mortem they exhibited small haemorrhagic lesions in the stomach, bowel, muscles and certain joints. The addition of orange, lemon or turnip (swede) juice to the diet failed to prevent, and in two of these (orange and lemon), actually hastened the onset of the symptoms, although cabbage exerted a protective action. Butter alone (as a source of vitamin A), failed to supplement a diet of soya meal and orange juice, although when used in addition to a salt mixture (Osborne-Mendel) it prolonged the lives of the animals, but did not afford complete protection against the poisonous effect of the meal. Guinea-pigs fed on soya meal with the addition of cod-liver oil, yeast, inorganic salts and orange juice also rapidly lost weight and died in 11-14 days.

The results of these feeding experiments conducted on guinea-pigs are in agreement with those obtained by Osborne and Mendel (40) and Daniels and Nichols (41) in similar experiments on rats.

(ii) Chemical and Biological.

Many samples of soya meal (oil extracted) were examined by the lead method and were found to agree very closely in regard to their chemistry, although they differed considerably in their content of the toxic substance.

The purified extract of each sample was first prepared by the usual method and was found to be very toxic when given hypodermically to animals.

A Rhesus monkey got 5 g. of this purified extract hypodermically and immediately became very weak in the legs. Later in the day it became much worse and was only able to move about with difficulty. The following day it was still very weak and lay on its side a great deal with its toes firmly flexed. It remained in this condition for 4 days after which it seemed to recover.

The "glucoside mixture" was then examined and was found to consist chiefly of sugars and inorganic salts. The yield obtained was usually 2-3 per cent. It was toxic however, and 0.2 g. (neutralised with sodium bicarbonate) given hypodermically to a frog produced paralysis for several hours, followed by increase of spinal reflexes lasting for several days. As in the case of the "glucoside mixture" of lathyrus, repeated treatment of this mixture with lead or mercuric acetate removed the poisonous constituent as an insoluble lead or mercury salt respectively. In one experiment 0.2 g.

of a "glucoside mixture," which had been treated twice with mercuric acetate and once with lead acetate, was given hypodermically to a frog and was found to have no action of any kind.

Examination by the usual method of the decomposition products derived from the lead acetate precipitate, showed the presence of an active principle, insoluble in strong alcohol or acetone, but soluble in water. It showed the same properties and gave the same chemical reactions as the active principle from lathyrus. It was further purified by treatment with sodium bicarbonate which "salted out" an organic complex of calcium and magnesium, which was filtered off and which, on being tested on frogs, was found to be inactive. On adding the filtrate to a large volume of strong alcohol a flocculent white precipitate of the sodium salt of the active principle was obtained which was filtered off and dried in vacuo over calcium chloride. The free acid was obtained from it by decomposition of its insoluble lead salt by sulphuretted hydrogen, followed by precipitation with strong alcohol. It has not yet however, been obtained in an absolutely pure state, despite many attempts to crystallize it from water and dilute alcohol.

The physiologically active principle of soya proved to be a white, amorphous, hygroscopic substance very soluble in water and very acid. It gave dense white precipitates with the salts of the heavy metals but no precipitate with the usual alkaloidal reagents. It did not give the reactions of a protein, amino-acid, glucoside or saponin. Para-nitro-phenacyl chloride and para-bromo-phenacyl bromide did not interact with it to form crystalline derivatives. So far, attempts to prepare a crystalline salt from it have failed but further efforts are being directed along these lines. Owing to the small yield obtained (.1 per cent.) it would almost require a miniature manufacturing plant to prepare the crude principle in any quantity for a thorough chemical examination. This is further indicated in view of the fact that the lead method, which is the only one at present available for its purification, results in an enormous loss of material (80 per cent.).

An examination of the alcohol (or acetone) used in the precipitation of this physiologically active principle showed the presence of large quantities of citric and phosphoric acids. The former was identified by the tests already given under lathyrus, and the latter by the ordinary analytical reactions for phosphates.

The citric acid crystallized from the alcohol (or acetone) in the form of rhombic prisms or aggregates of needles; the yield was usually 0.5 per cent. The action of these when tested (in the form of the sodium salt) on animals was found to be identical with that of the corresponding crystals from lathyrus.

The phosphate was recovered from the mother liquor by neutralising with sodium hydroxide and submitting the mixture to fractional crystallization. The yield of sodium phosphate obtained from the beans was usually 1 per cent.

Biological Experiments with Physiologically Active Principle.

The active principle proved to be very toxic when given hypodermically as a sodium salt to monkeys, rabbits and frogs. The following experiments were conducted by Professor Stockman (17b).

Monkeys.

A Rhesus monkey received 0.7 g. of the sodium salt of the active acid hypodermically and in a few minutes became very uneasy. It lay down a great deal owing to weakness in the limbs and all day remained very paretic and was unable to climb. Next day it was still weak and unable to climb; it ate very little food. On the third day it ran about freely but its legs were still weak. On the fourth day it seemed normal.

Rabbits.

A rabbit received 1 g. of the sodium salt of the active acid hypodermically and became very feeble and had marked weakness in its hind legs. Another animal which received 0.5 g. of the active acid neutralised with sodium bicarbonate showed no marked symptoms except a slight weakness of the hind legs on the day following the injection.

Frogs.

When 0.05 g. of the active acid (neutralised with sodium bicarbonate) was given hypodermically to frogs they immediately became paralysed, due to an action on the brain and spinal cord. The paresis usually lasted for several hours where the animals survived, and was followed by increased spinal reflexes lasting for several days. Some animals exhibited very marked muscular twitchings, others severe general tetanus. The general effect of a dose of 0.05 g. (neutralised) given hypodermically to a frog could be inhibited by the almost simultaneous injection of a dose of calcium acetate (0.03 g.)

Several experiments with the active principle were conducted by the writer on fish, but these did not indicate the presence of an active saponin. The ordinary minnow (Leuciscus phoxinus) and goldfish (Cyprinus auratus) were entirely unaffected by the presence of the principle in a dilution as low as 1 in 1,000 parts of water.

Numerous laking experiments conducted on defibrinated blood also showed the absence of an active saponin.

Other leguminous seeds, widely used for human food or cattle forage, were also investigated by the same methods and were found to be as toxic as lathyrus and soya. The poison in each of these proved to be analogous to, if not identical with, that already obtained from lathyrus and soya.

Lentils (Ervum lens).

This valuable pulse (Plate 1, Fig. 3) which according to De Candolle (42) is a native of western temperate Asia, Greece and Italy, is supposed to be one of the first plants cultivated by mankind. Lentils have been found in the lake dwellings of St. Peter's Island (Bronze Age). The red Egyptian lentil is cultivated in one or other variety in India, Persia, Syria, Egypt, Nubia, North Africa and in Europe along the coast of the Mediterranean and as far north as Germany, Holland and France.

Lentils are more peculiarly the food of the poor in all the countries where they are grown, and they are often spurned when better food can be obtained, hence the proverb, 'Dives factus iam desiit gaudere lente.' In India the lentil is eaten boiled as a dal, flavoured with various spices and condiments, also as a component part of the dish called kichri, and it is considered the most nutritious of the pulses.

In British commerce two kinds of lentils are principally met with, French and Egyptian. The former are usually sold entire, and are of an ash-grey colour externally and of a yellow tint within; the latter are sold like "split" peas, without the seed-coat and consist of the reddish-yellow cotyledons, which are smaller and rounder than those of the French lentil.

Haller says that lentils are so flatulent as to kill horses. Their bad reputation is ascribed to contamination with the seeds of the bitter vetch or tare lentil (Ervum ervilia), a plant which closely resembles the true lentil in many respects and whose seeds are without doubt possessed of deleterious properties — producing weakness or even paralysis of the extremities in horses which have been fed on them. The poisonous principle seems to reside chiefly in the bitter seed-coat, and can apparently be removed by steeping in water, since Gerard speaking of the bitter vetch says, "kine in Asia and in most other countries do eat thereof, being made sweet by steeping in water."

The herbage of the lentil is highly esteemed as green-food for suckling ewes and all kinds of cattle, being said to increase the yield of milk.

The present investigation was conducted on ordinary lentils as sold in market-places and has shown (1) that this pulse exerts an action similar to lathyrus when fed to monkeys and (2) that it contains an acid substance which is similar in its action to that found in lathyrus and which can be extracted by a similar chemical method.

Experimental.

A preliminary feeding experiment showed that a monkey fed on a diet of lentils cooked by steaming, with the addition of some milk and a plentiful supply of fruit daily, gradually lost its normal activity and exhibited slight muscular tremors.

On the 49th day it became very paralysed and remained in this condition until its death on the 52nd day.

A post-mortem examination showed the viscera to be healthy but on microscopic examination the brain and spinal cord showed degenerative changes similar to those found in the lathyrus monkey.

Chemical and Biological.

The present investigation consisted of the examination of the cold water extract of one sample (2 kilos.) of ground lentils by the usual lead method.

The purified extract was found to be toxic and the following animal experiments were conducted with it.

Monkeys.

A Bonnet monkey, which received hypodermically 4 g. of the purified extract, sat huddled up all day and lost its normal agility. Two days later it received 5 g. hypodermically and became much weaker. A day later, a further 2 g. was given and almost immediately it became very paralysed. It exhibited clonic spasms of the arms and general clonic convulsions with marked flexion of the joints (Plate 7). These passed off but the animal still remained very paretic. The following day it continued to be very weak with flexion of the fingers and on the third day it seemed normal again.

Lentils.

PLATE 7.

Monkey after hypodermic injection
of purified extract.

Rabbits.

A rabbit received 4 g. of the purified extract hypodermically but showed no symptoms of any kind.

Decomposition of the lead acetate precipitate yielded an acid substance, insoluble in alcohol, which had an action on frogs similar to that of the corresponding substance from lathyrus and soya. The yield was 0.08 per cent. It showed the same properties and gave the same chemical reactions as the active principles already isolated. 0.05 g. of this substance (neutralised with sodium bicarbonate) given hypodermically to a frog produced paralysis for several hours, followed by increase of spinal reflexes lasting for several days. Larger doses caused death, and smaller doses depression followed by increase of reflexes.

The small amount of material available (1.4 g.) did not permit of further investigation.

The Common Pea (Pisum sativum).

The common, cultivated or garden pea (Plate 2, Fig. 3) is the seed of Pisum sativum. Its origin is not known, and since it has not been found in a wild state, it is considered that it may be a form of Pisum arvense. It was known to Theophrastus (43), and De Candolle (42) points out that the word 'pison' or its equivalent occurs in the Albanian tongue as well as in Latin, whence he concludes that the pea was known to the Aryans and was perhaps brought by them into Greece and Italy. Peas have been found in the Swiss lake dwellings of St. Peter's Island (Bronze Age). Since they constitute a highly nutritious article of diet owing to their large content of protein matter and are easily cultivated, they are grown to some extent in most countries, but more extensively in India, France, Italy, Canada and South America. In India the green pods are collected while the plant is growing, and are either cooked and eaten like French beans, or the young seeds are extracted and eaten in the same way as by Europeans.

The peas, when fully ripe may have either a yellow or green colour, and are imported into this country in large quantities from many parts of the world.

The present investigation was conducted on both dried green peas ("blue" peas) and Chilian peas (yellow) and has yielded results similar to those found for lathyrus and soya etc.

Experimental.

A preliminary feeding experiment showed that a monkey was unaffected after 30 days' feeding on a diet of "split" peas (Chilian) cooked by steaming with the addition of milk and fruit daily. It was then given daily, drinking water in which peas had been macerated and gradually became paretic. It remained in this condition until it died on the 56th day from the commencement of the experiment. A post-mortem examination showed pathological changes in the brain and spinal cord similar to those already described (for lathyrus, soya etc.).

The aqueous extracts of two separate lots of peas were examined by the usual lead method. They yielded active purified extracts one of which (from yellow "split" peas) was tested on a monkey by Professor Stockman (17b). It proved to be very poisonous, since 4 g. given hypodermically produced paralysis followed by death in 7 days. After death the animal showed the usual degenerative changes in the brain and spinal cord.

Decomposition of the lead acetate precipitates by the usual method yielded an alcohol-insoluble acid substance which was very toxic when tested on frogs. The yield however always proved to be very small; in one experiment .95 g. was isolated from 2 kilos. of Chilian peas. A dose of .05 g. (neutralised with sodium bicarbonate) given hypodermically to a frog produced complete paralysis almost at once, followed by death in 3 hours. A smaller dose produced paralysis, but the frog recovered in 24 hours when it exhibited marked increase of reflexes. The active principle possessed the same physical properties and showed the same chemical reactions as the corresponding substance from lathyrus and soya.

Haricot Bean (Phaseolus vulgaris).

Haricot bean (Plate 2, Fig. 4), is the name given to the seed of many varieties of Phaseolus vulgaris, a plant which is supposed to have been indigenous to South America, and which is now cultivated in many parts of the world where the climate and soil are suitable. After being cooked the beans are wholesome and nutritious as an article of food, although McDougall (21) has recorded cases of illness resulting from their ingestion in the raw state. Owing to their high content of protein matter they have been frequently recommended as substitutes for the more expensive sources of protein, viz., meat, milk and eggs.

The genus Phaseolus, to which this bean belongs, includes the Java or Rangoon bean (Phaseolus lunatus), which is imported into this country in considerable quantity for stock-feeding, and has at times caused the death of a large number of animals. It also includes the Mung bean (Phaseolus Mungo), which Watt (5) records as the most esteemed of all pulses in India.

The beans have not aroused any marked suspicions that they might be poisonous, although Professor Mellanby (69b) in 1931 showed that a dog fed on a supplemented diet of dried haricot beans developed an abnormal gait, and after death showed pathological changes in the spinal cord. The addition of cod-liver oil to this diet prevented similar changes in another animal.

The present investigation has shown for the first time that the beans contain (1) a principle of acidic character which is poisonous to animals on hypodermic or intravenous injection and (2) citric acid.

Experimental.

No preliminary feeding experiments were conducted.

Several samples of the ordinary beans sold in market-places were examined and were found to exhibit considerable differences in their toxic content.

Decomposition of the lead acetate precipitates by the usual method yielded an acid principle, insoluble in strong alcohol, which was found to be toxic when tested on rabbits and frogs, and which gave the same chemical reactions as the corresponding acid substance obtained from the other leguminous seeds. The yield was .05 per cent.

The alcohol used in the precipitation of this principle was found to contain phosphoric and citric acids. The organic acid crystallized on setting aside for some days the alcohol which had been concentrated to a thin syrup, and was identified by micro-chemical methods. The yield obtained, however, was usually small (.2 per cent.) but probably represented only a fraction of the total amount present in the beans.

The following experiments were conducted on rabbits and frogs with the physiologically active principle.

Rabbits.

A rabbit (1930 g.), which received 1 g. of the crude active principle (neutralised with sodium bicarbonate) into a vein of the ear, went into convulsions and died almost immediately.

Another animal (1940 g.), which received .5 g. of the active substance (neutralised with sodium bicarbonate) intravenously, showed muscular tremors and weakness in the legs.

A third animal (1940 g.) got .21 g. (neutralised) intravenously but did not show any marked symptoms.

Frogs.

Doses of 3 to 5 cg. of the active principle (neutralised) when given hypodermically to frogs produced paralysis and at times tetanus, followed by increase of spinal reflexes.

The Vetch (Vicia sativa).

This small pea (Plate 2, Fig. 1) which has a black seed-coat, is the seed of Vicia sativa, a plant which is indigenous to Great Britain, and has from a remote period, been grown in Southern and Central Europe as a forage-plant. It is also called the tare, and is mentioned in the Authorized version of the Bible (44) although some botanists think that the Darnel (Lolium temulentum) is meant. Some of the older English writers combine both names in the termination tare-fytche.

In India it does not occur truly wild, but always cultivated, though it is said to occur spontaneously in the Patna district where, though very little used or esteemed, the seeds are made into cakes by the poor.

The present investigation has shown the presence in the peas of an active principle which is similar in properties to that obtained from lathyrus.

Experimental.

No preliminary feeding experiments were conducted.

The aqueous extract of one small lot (2 kilos.) of Russian tares was fully examined by the usual lead method.

The purified extract (yield 2 per cent.) was found to be toxic and the following experiments were conducted with it by Professor Stockman (17b).

Monkeys.

A monkey received 4 g. of the extract hypodermically and immediately became weak and paretic. The effect lasted for 24 hours.

Rabbits.

A rabbit (650 g.) got 4 g. of the extract hypodermically but did not show any marked symptoms.

There was isolated (by the usual method), from the decomposition products derived from the lead acetate precipitate, a crude acid principle (yield ·1 per cent.), insoluble in absolute alcohol, which was active when tested on animals. It possessed the same physical properties and gave the same chemical reactions as the corresponding acid substance from the other leguminous seeds. The following experiments with its sodium salt were conducted by Professor Stockman (17b).

Monkeys.

A monkey received hypodermically ·95 g. of the sodium salt and immediately became very paretic. This condition lasted for two days but on the third day the animal was almost normal again.

Rabbits.

A rabbit (1300 g.) which received hypodermically 1.2 g. of the sodium salt immediately became paretic and lay on its abdomen. The paresis lasted for 2 hours, after which the animal seemed to recover.

The Pigeon Pea (Cajanus indicus).

This small pea (Plate 2, Fig. 2), which has a shiny orange-brown seed-coat, is the seed of a plant very extensively grown in Africa, Madagascar and in the East and West Indies. It was probably introduced into the West Indies and America from Africa, where several botanists have found it growing in the wild state. It has been long cultivated in India on a large scale, but nevertheless, no Indian botanist has ever recorded having found it wild, or even naturalised there; thus there would seem little doubt that in India, it is an introduced plant. It is not mentioned in any of the Sanskrit works.

Although the peas are not much used in this country, except for feeding pigeons, they are largely consumed by all classes in many parts of India, and in some places, they are eaten to a larger extent than any other pulse. They are known to the Hindus as arhar (urhur), and are thus used:

(1) Parched in hot sand and eaten dry, or with salt or oil.

(2) Ground into flour and made into cakes, or ground into meal and mixed with water.

(3) Steeped in cold water and rubbed into a paste, which is made into balls; these are dried in the sun and used in curries.

(4) Boiled in about six times their bulk of water until soft, and then flavoured with condiments.

The raw or cooked peas have always been regarded as very wholesome and nutritious, although Kirk (45) records that they have been known to cause chronic poisoning in man. He gives as the symptoms, urticaria, heat in the stomach, discoloration and affections of the skin (not unlike those occurring in pellagra) and rheumatic pains.

The present investigation has shown that this pea contains an active principle which is poisonous to animals on hypodermic injection.

Experimental.

No preliminary feeding experiments were conducted.

The cold water extract from a small sample (1 kilo.) of the ground peas was fully investigated by the lead method.

The purified extract was first examined and was found to be very toxic when given hypodermically to frogs, but only very slightly so to rabbits. No experiments were conducted on monkeys.

Rabbits.

A rabbit (800 g.) received 2 g. of the extract (neutralised) hypodermically and showed little or no symptoms except slight depression. On the following day it seemed normal and observations were then stopped.

Frogs.

Doses of 1 to 2 dg. of the purified extract (neutralised) given hypodermically to frogs produced paralysis for several hours or a day, followed by increase of reflexes lasting for several days.

By the usual method, there was isolated from the decomposition products derived from the lead acetate precipitate, a minute amount of an active principle insoluble in strong alcohol. This was poisonous when given to frogs as a sodium salt, doses of 2 to 3 cg. producing paralysis followed by increase of spinal reflexes. The yield obtained (.15 g.) however did not permit of a fuller examination of its pharmacology. Micro-chemical methods of analysis showed that it gave the same chemical reactions as the corresponding substance from the other leguminous seeds.

PART II.

CEREALS.

Cereals.

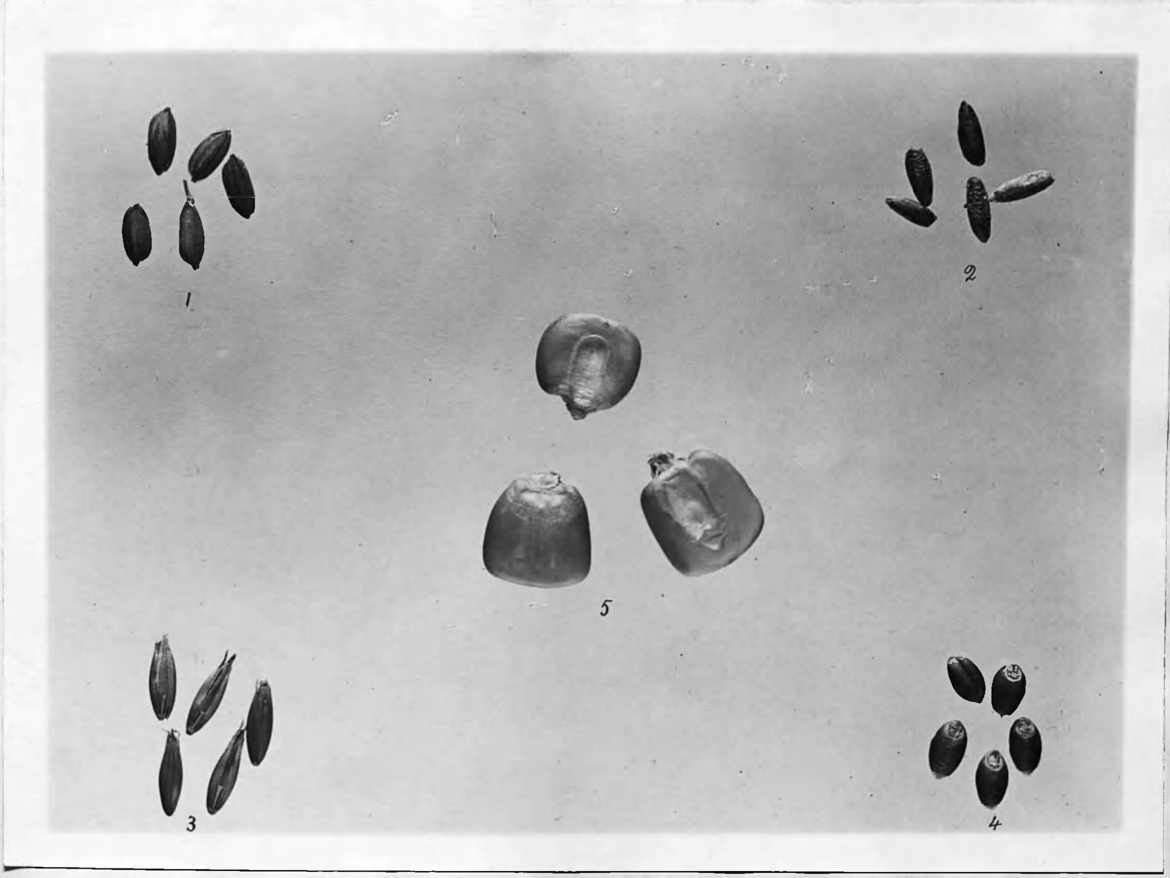


PLATE 8.

Fig. 1. Rice grains with husk ("paddy").

Fig. 2. Rye grains.

Fig. 3. Scotch oats.

Fig. 4. Wheat (grown in Scotland).

Fig. 5. Maize (East African).

Cereals.

General. Cereals are the fruits of the cultivated grasses which belong to the natural order Graminaceae. As a class, they are characterized by a considerable and often excessive proportion of starch. The quantity of protein matter is usually low compared with the pulses, and ranges from 18 per cent. in the hardest wheats to 6.5 per cent. in the poorest rices. The oil is largely present in the coats and embryos of the grains. The mineral matter or ash varies from .6 per cent. in polished rice, to 2 per cent. in wheat.

The following table gives the mean composition of the seven principal cereals used by mankind (viz., rice, wheat, maize, millet, oats, rye and barley).

Mean Composition of the Cereals.

	Mean.	Limiting Figures.
Water	12.4 per cent.	11.6-12.8 per cent.
Protein	10.3 " "	7.3-13.5 " "
Carbohydrate	69.7 " "	56.0-78.4 " "
Fat	1.8 " "	.6-3.6 " "
Fibre	4.0 " "	.4-16.6 " "
Ash	1.7 " "	.6-2.3 " "

Maize (Zea Maÿs).

Maize (Plate 8, Fig. 5) is the fruit of a cultivated grass which according to Darwin (46) and De Candolle (42) was indigenous to Mexico and Peru, and was brought to Europe in 1500 by the Spaniards and later by the Portuguese. It is now very extensively cultivated as a foodstuff for man and domestic animals in most countries where the conditions are suitable. The following statistics for 1932 show the relative importance of maize in the countries mentioned.

The relative importance of maize and other cereals.

	Acres under maize.		Total acres under wheat, barley, oats and rye.	
Italy	3,622	Thousand	14,580	Thousand.
Roumania	11,775	"	14,317	"
Hungary	2,877	"	7,211	"
Jugo-Slavia	6,442	"	4,480	"
Czechoslovakia	12,713	"	8,412	"
Argentine	11,623	"	26,587	"
U.S.A.	107,729	"	112,885	"
U.S.S.R.	9,084	"	203,149	"

It is also fairly extensively grown in India where it is largely consumed made into cakes or porridge (lapsi or gathá), or parched in hot sand and eaten with gúr or salt. In America, it continues to be an important article of diet, since in one or other form it is used in all parts of the country among all classes of the people.

The grain is largely imported into this country from the Argentine, German East Africa and British South Africa; it is used for feeding stock and poultry, and in the manufacture of starch and corn flour, which involves the separation of the oil. The latter is used for cooking purposes, and the residual cake, mixed with other articles of food, for feeding stock.

Although maize is rich in carbohydrates (70 per cent.), it is not considered so wholesome and nourishing as wheat, since it is somewhat deficient in proteins (9.5 per cent.). Nevertheless it forms a perfectly harmless food to man or animal when used even as a considerable part of a mixed dietary, although gross over-consumption, especially if combined with a very limited variety and supply of other foods, gives rise to pellagra. It is evident that this disease will be most likely to fall on members of the poverty-stricken classes in certain countries, whose food consists almost exclusively of maize.

Pellagra.

The name 'pellagra' (Italian pelle agra, rough skin) was introduced into medical literature by Frapolli (47) in 1771, to describe a peculiar disease of comparatively modern origin. The disease, which is characterized by easily recognised symptoms, was apparently unknown to the physicians of antiquity. Its scientific history dates from the treatise written by the Spaniard, Gaspar Casal (48) in 1735 which was not fully published until 1763. It is interesting to note that Casal, in this work, actually ascribed the disease (mal de la rosa) to faulty nutrition. For some time pellagra was supposed to be practically confined to the peasantry in parts of Italy (especially Lombardy), France (Gascony), Spain (the Asturias), Roumania and the Balkans; but it has been identified on a smaller scale in various parts of the British Empire (Barbadoes, India), Egypt and South Africa. In the United States of America sporadic cases have been noted from 1864 when Gray and Tyler first definitely observed it; but since 1907 it has increased to alarming proportions. Funk (49a) states that in America between 1907 and 1912, 20,000 persons died of pellagra, the mortality being 4 per cent. among those suffering from the disease. It is in Italy and Roumania however that the disease has been most prevalent.

Factors concerned in the Geographical Distribution of Pellagra.

From the date of the earliest observations on pellagra in Spain, down to the present day, the disease has been ascribed to the use of maize as food, and this view of its mode of production is now almost universally held. It is based on the facts that the disease did not appear in Europe until after the introduction of maize as an article of food, that it generally disappears when maize is excluded from the dietary, and that it is endemic only in those areas where this cereal is very largely consumed. The history of the rise of the disease in any country, usually shows that about a generation after the introduction of maize as an article of food, sporadic cases of pellagra arise. Its relative frequency, moreover, in the different parts of those countries where it is met with, seems to depend on the extent to which maize or maize products are eaten by the people. In Italy this is especially the case, and pellagra is there most common where polenta or maize porridge is the staple food of the peasants, and least so where this is largely combined with or replaced by other articles of food, such as wheaten bread, potatoes and chestnuts. In Egypt, again, pellagra is very common in Lower Egypt where maize is much grown and eaten, and slightly rarer (although still very prevalent) in Upper Egypt where millet tends to displace maize as the chief grain food of the peasants. Although the disease has only been known for about 80 years in Egypt, it has now become the greatest cause of insanity and of deaths among the insane.

Symptoms.

The malady is essentially chronic in character. The indications usually begin in the spring of the year, declining towards autumn, and recurring with increasing intensity and permanence in the spring following. A peasant, who is acquiring the disease, feels unfit for work, suffers from headache, giddiness, singing in the ears, burning of the skin, especially in the hands and feet, and diarrhoea. At the same time a painful red rash appears on the skin which smarts on direct exposure to the sun. With each successive year the patient becomes more like a mummy, his skin shrivelled and sallow, or even black at certain spots, his angles protruding, his muscles wasted, his movements slow and languid and his sensibility diminished. After a certain stage the disease passes into a profound disorganization of the nervous system; there is a tendency to melancholy, imbecility and a curious mummified condition of the body.

Slight and early cases recover readily if the diet is changed in time, but in all others the prognosis is bad.

After death a general tissue degeneration is observed. In the chronic cases there is scattered degeneration of the nerve fibres in the spinal cord and peripheral nerves. Degeneration has also been met with in the cells of the spinal ganglia, posterior cornu and Clarke's column. In these the Nissl bodies and the fibrils disappear, while the nucleus is placed excentrically and often peripherally. Slight diffuse sclerosis in the system tracts of the cord and medulla may also occur.

Aetiology.

The exact cause of pellagra is at the present time unknown in spite of the fact that numerous "theories" have been advanced from time to time to explain its aetiology. These have generally but not invariably incriminated maize as its cause. Antoniu (50) and Goldberger and Wheeler (51) have shown that the general consensus of opinion is correct, by producing the symptoms of pellagra in men fed wholly or partially on maize diets. Many "theories" have been advanced to explain the precise mechanism of the maize; the deficiency (protein, amino-acid or vitamin), contamination and infection (fungoid or parasitic) of the grain have from time to time been blamed. None of these however has withstood the test of aetiological inquiry.

The present investigation, which was conducted in collaboration with other workers, has shown that maize contains an acid substance, which is poisonous to monkeys and other animals, and which can cause degenerative changes in the brain and spinal cord when given per os or hypodermically in the form of a neutral body. These pathological changes are identical with those found in monkeys fed on maize diets and in pellagra.

It will be evident that without generalizing further than the observed facts warrant, a big advance in knowledge has been made concerning the cause of pellagra. However it should always be understood that studies made upon animals cannot be accepted unreservedly in the domain of human physiology since they are merely tentative and introductory in nature, and must await confirmation by clinical medicine.

Experimental.

(i) Preliminary feeding experiments.

Monkeys.

Monkeys exhibited very different degrees of susceptibility to maize feeding, and when attempts were made to reinforce their rations by adding drinking water, in which meal had been macerated, they usually refused to eat their food. In one case a monkey ate a full meal of maize porridge and was found dead next morning. (After death no lesions of any kind were discovered.) This was evidently a case of acute maize poisoning, but generally the symptoms were more chronic. The following is a typical case.

A Bonnet monkey was fed on maize cooked by steaming, supplemented with butter (10 g.), milk (2 oz.) and a plentiful supply of fruit daily. It gradually lost weight, became weaker and after 44 days died from gradually deepening paralysis. After death it showed ~~the~~ typical widely-spread degeneration of the nerve fibres and injury of the nerve cells, in both the cerebro-spinal and sympathetic systems. (Plate 9).

Another monkey was fed on a similar diet supplemented with 5 grains of calcium lactate and 10 grains of sodium bicarbonate, and showed no symptoms even after 9 weeks' feeding. The addition of the salts seemed to counteract the effect of the poisonous substance in the maize.

PLATE 9.

Section showing degenerative changes in oculomotor fibres of monkey fed on maize diet (Marchi's technique).

Rabbits.

Rabbits also varied greatly in their susceptibility to maize feeding, but generally young rabbits, fed on maize porridge with a plentiful supply of cabbage, lost weight and failed entirely to thrive. During the fortnight preceding death, which usually ensued in 3-4 months, they lost much of their hair, were greatly emaciated, and exhibited paresis and tremors of the legs. The addition of calcium acetate to this diet failed to prevent the death of young animals.

Mature rabbits on the other hand, were generally only slightly affected, or not at all, by the diet of maize porridge and cabbage. Addition of calcium salts (lactate or acetate), cod-liver oil or both, did not seem to influence the effect of the diet in any way. In one case an adult rabbit fed on a diet of maize porridge (ad libitum), plus cabbage (70 g.) and calcium lactate (½ g.) became greatly emaciated, exhibited dermatitis on the legs, and eventually died from gradually deepening paralysis after 3½ months' feeding. Another rabbit on the same diet, throve and was in perfect health 125 days after the commencement of the experiment.

The offspring from rabbits fed on maize diets, were usually undersized and did not show the normal rate of growth. In some cases the mothers failed entirely to rear their young.

After death an analysis of the bones of these rabbits showed them to be greatly depleted of mineral matter, especially calcium salts. This is in agreement with the observation of Weiske (52) concerning rabbits fed on oats.

Guinea-pigs.

Guinea-pigs fed on a diet of maize porridge plus orange juice lost much hair, became greatly emaciated and finally died after 10-14 weeks. Usually during the week preceding death, they exhibited marked paresis and weakness in the legs. The addition of calcium acetate, sodium bicarbonate, or cod-liver oil did not alter in any way the effects of the diet. Cabbage (ad libitum) on the other hand, exerted a protective action and allowed animals to thrive on a maize diet, where formerly they died, the inference being that the cabbage maintained the acid-base equilibrium of the blood, which had been disturbed by the prolonged daily ingestion of the acid substance present in the maize.

(ii) Chemical and Biological.

The present investigations were conducted on the meal from both yellow and white maize, and also on the maize germ cake which is largely used for feeding stock in this country, and which is the residue of the whole grain after the starch (corn flour) and oil have been extracted.

In the beginning of the research, attempts were made to extract the active principle with hot 25 per cent. alcohol. This method was finally abandoned, partly owing to the fact that the feeding experiments seemed to show the solubility of the poisonous substance in cold water, and partly owing to the difficulty experienced in obtaining clear extracts (free from starch).

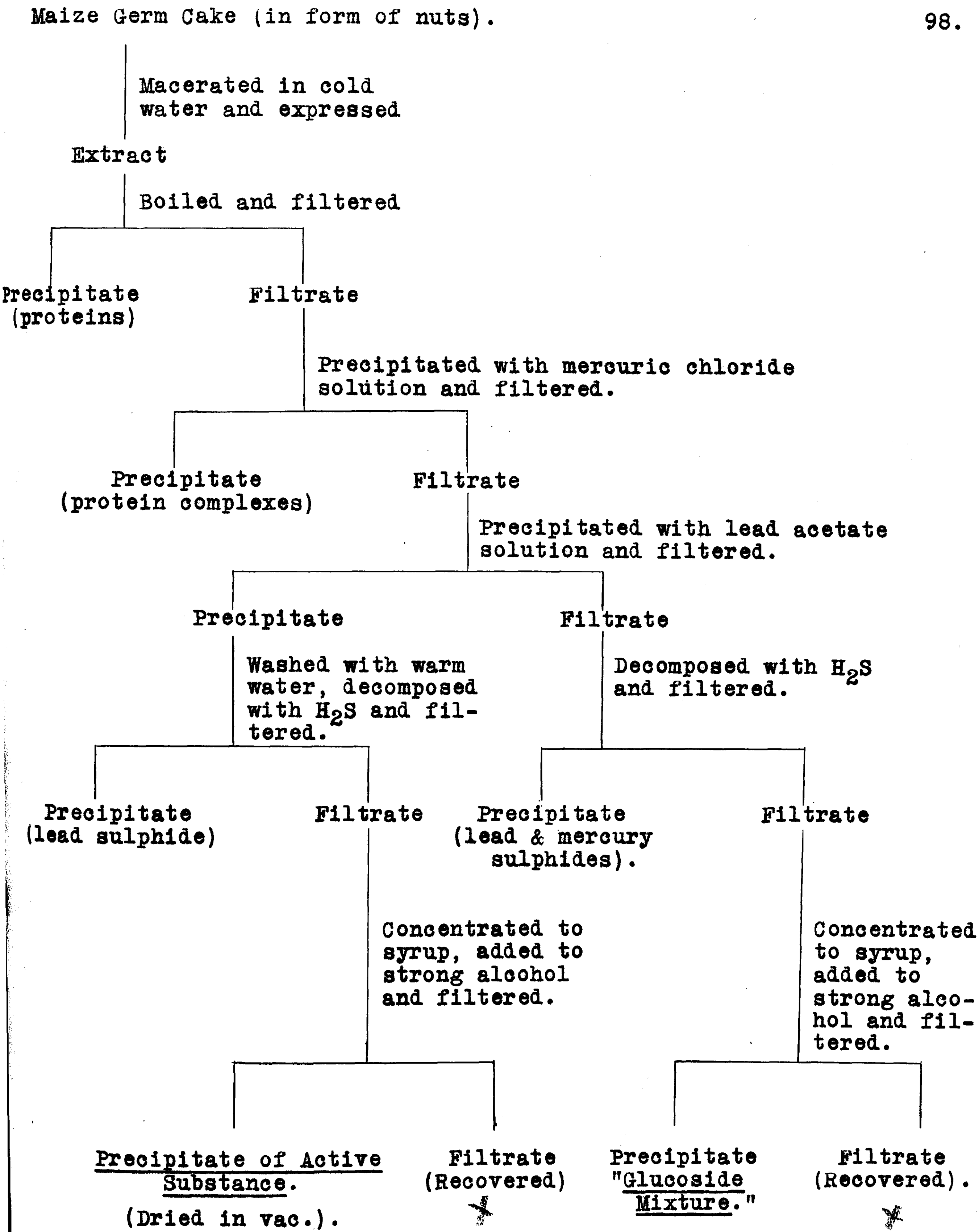
Next the writer's attention was turned towards the isolation of the fluorescent substance "zeochin," which is described by Suarez (53), and which is supposed to act photo-dynamically on red blood corpuscles. This offered a fruitful field of inquiry, since the work of Raubitschek (54) has tended to show that an exclusive maize diet, good or bad, proves deleterious to white mice and guinea-pigs when these animals are exposed to sunlight. It is maintained by this worker that photo-dynamic substances are introduced by the cereal into the blood, and these under the influence of sun-light become toxins, and thus cause inflammation of the skin and other symptoms resembling pellagra. Following the directions of Suarez however, it was found impossible to isolate even a crude active product, and the attempt was finally abandoned. In this connection, it is interesting to record that in this investigation several rabbits on maize diets exhibited marked lesions of the skin (dermatitis), even when they were not exposed to direct sunlight. It seems highly probable that the skin lesions found in pellagra are due to a tropho-neurosis, which however renders the skin extraordinarily sensitive to the irritant effects of sunlight.

Attempts were then made to extract any poisonous alkaloids which might be present in fresh maize, since other workers had found minute traces of these in stale maize bread. The Stas-Otto method for the detection of alkaloids however, showed the absence of these in the ordinary grain.

Finally, in view of the success attained in the investigation of the leguminous seeds, the lead method was applied to the cold water extract of the maize germ cake, and the following scheme which was later applied to the other cereals was adopted (Page 98).

The investigation was conducted on the cold water extract from 56 lbs. of maize germ cake.

The decomposition product derived from the lead acetate precipitate yielded, by the usual method, 50 g. of a crude physiologically active principle insoluble in strong alcohol. This proved to be a white, amorphous, hygroscopic substance, very soluble in water and very acid. It dissolved in very dilute alcohol, but was insoluble in cold and boiling ether, acetone, chloroform, benzene and trichlor-ethylene. It gave precipitates with lead acetate (ordinary and sub), mercuric acetate, calcium chloride, sodium hydroxide, ammonium hydroxide and sodium bicarbonate, but no precipitate with the usual alkaloidal reagents or ammonium sulphate. When tested for protein, amino-acid, glucoside or saponin it gave negative results. Attempts were made to crystallize it from water or dilute alcohol, but these so far have resulted only in the formation of a glassy mass. It was finally further purified by dissolving in water and neutralising with sodium bicarbonate which "salted out" a considerable


Maize Germ Cake (in form of nuts).
Macerated in cold water and expressed
Extract
Boiled and filtered
Precipitate (proteins)
Filtrate
Precipitated with mercuric chloride solution and filtered.
Precipitate (protein complexes)
Filtrate
Precipitated with lead acetate solution and filtered.
Precipitate
Washed with warm water, decomposed with H_2S and filtered.
Filtrate
Decomposed with H_2S and filtered.
Precipitate (lead sulphide)
Filtrate
Precipitate (lead & mercury sulphides).
Filtrate
Concentrated to syrup, added to strong alcohol and filtered.
Concentrated to syrup, added to strong alcohol and filtered.
Precipitate of Active Substance.
(Dried in vac.).
Filtrate (Recovered)
Precipitate "Glucoside Mixture."
Filtrate (Recovered).

quantity of an organic complex of calcium and magnesium, which was filtered off and which, on being tested on frogs, was found to be inactive. On adding the filtrate to a large volume of strong alcohol a flocculent white precipitate of the sodium salt of the active principle was obtained, which was filtered off and dried in vacuo over calcium chloride. The free acid was obtained from it by the decomposition of its insoluble lead salt by sulphuretted hydrogen, followed by precipitation with strong alcohol. This method however, resulted in a very considerable loss of material owing to absorption on the lead sulphide precipitate. The purified principle gave the same reactions as the crude body, but little or no precipitate with alkalies. A combustion analysis yielded the following results:-

C = 25.10 per cent.

H = 1.82 per cent.

N = 1.49 per cent.

The alcohol used in the precipitation of the crude physiologically active principle, was found to contain only phosphoric acid and a trace of the active principle itself. The former yielded 40 g. of crystalline di-sodium hydrogen phosphate, on neutralisation with sodium hydroxide followed by crystallization. Although the phosphate present was isolated as the sodium salt, it seems highly probable that it exists in the maize grain as the acid salt of potassium (KH_2PO_4).

The "glucoside mixture" was found to be inactive, or nearly so, when tested on frogs, while chemical examination showed it to consist chiefly of sugars and inorganic salts.

A sample (10 kilos.) of pure maize germ was also examined by the same method and was found to contain the same physiologically active principle, but the yield (15 g.) was much smaller than that obtained from the same quantity of the maize cake.

Biological Experiments with the Physiologically Active Principle.

This substance, on being tested on monkeys and frogs, was found to be very toxic on hypodermic injection. Drs. Johnston and Stockman have shown in an article in course of publication that it is also poisonous to monkeys when given per os, repeated administrations of small doses resulting in the production of degenerative changes in the nervous system, identical with those found in monkeys fed on a maize diet and in pellagra.

Monkeys.

A small monkey received per os, in doses of 2-5 g. at intervals of 1-4 days, 19 g. of the sodium salt of the active principle without showing any marked symptoms, except some slight depression and carpopedal contraction. This animal proved rather refractory to oral administrations however, since it usually vomited at least half of the dose, and was apparently able to detoxicate the rest in the body. It then received 3 g. of the salt hypodermically and in 10 minutes lay on its side; occasionally it collapsed and lay flat on its abdomen. It was apparently very paretic and remained in this condition during the rest of the day. The following day it was still very helpless and paretic; it moved about with difficulty and was hardly able to climb. A large phlegmon formed at the point of injection owing to a thickening of the skin; examination showed the presence of little or no sero-sanguineous fluid. On the third day the animal was still weak and was then killed by chloroform.

After death it showed degenerative changes in the brain and spinal cord identical with those found in the monkeys fed chiefly on maize and in pellagra. There was also some slight haemorrhage in the brain.

Frogs.

When .05 g. of the sodium salt of the active principle was given hypodermically to a frog it immediately became paralysed and gave no response to pinching. This effect lasted for several hours and was followed by increase of spinal reflexes lasting for several days. Another animal received a similar dose and immediately became paretic. It showed marked muscular twitchings which lasted throughout the day; at times it exhibited severe tetanic spasms of the legs. The paresis lasted for three days, but on the fourth day it was found dead. All the muscles and nerves however were excitable to the faradic current.

The administration first of .03 g. of calcium acetate hypodermically to a frog, exerted a protective action against the effects of a hypodermic injection of .05 g. of the sodium salt. Similarly a frog which received hypodermically .05 g. of the salt, followed immediately by .03 g. of calcium acetate, did not show any marked symptoms except a very slight increase of reflexes.

These experiments on frogs seem to indicate that the active principle affects the calcium metabolism of the body, while the writer has already shown that a monkey fed on a supplemented maize diet with the addition of a compensatory salt mixture, was unaffected even after 9 weeks' feeding. In this connection it is interesting to record that Nicolaidi (55) has shown that pellagra is especially characterized by a disturbance in the mechanism of nutrition, which manifests itself through considerable demineralization in the organism, resulting in a surprising increase in the amount of mineral elements eliminated in the faeces.

Conclusion.

The investigation has shown that maize contains an active principle, which on being administered hypodermically or per os to monkeys, produces symptoms and pathological changes in the nervous system identical with those found in animals fed chiefly on this cereal and in pellagra. The precise mode of action of this principle is at present unknown but it seems to have a primary effect as an irritant poison and also a secondary effect, inasmuch as it tends to upset the mineral metabolism of the body by depleting the blood and tissues of their inorganic salts, especially calcium. The large content of acid phosphate in maize probably also plays an important part in this disturbance of the mineral metabolism of the body.

Other common cereals were also examined by the same method and were found to contain either the same acid substance or a similarly acting body. This probably explains the number of sporadic cases of pellagra which have been ascribed to the use of cereals other than maize.

Rice (Oryza sativa).

Rice (Plate 8, Fig. 1) is the grain of a cultivated grass, which according to Roxburgh (56) has originated from a wild plant, called in India Newaree or Nivara, which is indigenous to India and tropical Australia. It is extensively cultivated in India, China, the Indian Archipelago, West Indies, Central America, the United States and some of the southern countries of Europe. The following table shows the extent to which it is grown in the East.

Production of Rice (1926).

	Tons.
British India	34,000,000
Japan	6,200,000
Korea	2,700,000
Indo-China	3,700,000
Dutch East Indies	3,200,000
Siam	2,800,000

Rice has long been the chief article of diet in the greater part of Asia, and it has been estimated that roughly 600 millions of the inhabitants of the earth subsist on it, either alone, or in combination with other articles of diet. When supplemented with peas and cereals of all kinds, it has proved a highly nourishing food, but gross over-consumption, especially if combined with a very limited variety and supply of other foods, has given rise to beri-beri. However, the number of people contracting this disease is a very insignificant fraction of the total population subsisting on rice as a staple article of diet.

Gross over-consumption of rice is also known to cause pellagra, although the occurrence of this disease amongst rice-eaters is very rare. In recent years 70 cases have been reported in Japan, and a smaller number in China, in persons of the poverty-stricken class whose staple diet was rice. Viswalingam (57) has described an outbreak among Chinese coolies in the Malay States who were fed on a diet of polished rice, fish, pork, potatoes and green vegetables. Their dietary did not include maize in any form.

The present investigation which was conducted on several samples of rice, has shown the presence of very minute amounts of acid substances, which proved to be poisonous when tested on frogs.

Experimental.

(i) Preliminary Feeding Experiments.

Monkeys.

Attempts to produce the symptoms of beri-beri or pellagra in monkeys met with varying amounts of success. This seems to be due to the fact that the rices sold in the European market vary greatly in their toxicity, and contain either no poisonous principle, or at most, only a very small amount which would exert no deleterious effect in a mixed dietary. In one experiment two monkeys were fed for 4-5 months on ordinary polished rice (cooked by steaming) with the addition of orange juice, without showing any marked effects except loss of weight. Schaumann (58) found that a monkey fed on rice lost appetite and developed a paralysis of the lower extremities and progressive marasmus, while Gibson (59) showed that a monkey fed on polished rice with the addition of a compensatory salt mixture, became oedematous (especially in the face) and died in 42 days.

In another experiment a monkey received in addition to its food (polished rice), drinking water in which some Carolina rice had been macerated and immediately became much weaker. Further experiments with this sample of grain undoubtedly showed that it contained something deleterious to monkeys. Similar results were obtained with another sample of Rangoon rice. These experiments showed the water-soluble nature of the toxic substance or substances. After death, this monkey showed marked degenerative changes in the cells of the spinal cord and root ganglia.

Pigeons.

Several feeding experiments were conducted on pigeons in attempts to counteract the effect of the acid substances which were shown to be present in the rices. The effect of the addition of salt mixtures and the ash from rice polishings to rice diets, which were known to cause polyneuritis, were also investigated, since it was shown by chemical analysis that rice polishings were very rich in mineral matter (especially calcium). Scala (60), Kilbourne (61) and others have advanced the hypothesis that polyneuritis in fowls originates in a deficient mineral nutrition.

Analysis of Rices.

	Mean per cent. Ash.	Mean per cent. Calcium in Ash.
Siam Rice	.465	.012
Ballam Rice	.706	.045
'Parboiled' Rice	.720	.030
Rice Polishings	10.12	.286

These experiments were in all cases unsuccessful and the pigeons died of polyneuritis gallinarum, unless their diets were changed. Only those substances which are known to contain the water-soluble vitamin B complex were able to prevent the death of affected birds. It may be that the failure of various mineral substances (especially those from the rice polishings) to bring about appreciable benefit is explained on the ground of inability to reproduce the exact complexes found in the original food material.

Experiment 1.

Two pigeons fed on polished Patna rice (raw) gradually lost weight and one became polyneuritic (Plate 10, Fig. 3) and died after 25 days' feeding; the other recovered when its diet was changed to rye, after having lost nearly half its body-weight in 35 days.

Experiment 2.

Two pigeons fed on polished Patna rice (60 g.) plus rice bran (6 g.) gradually lost weight and one died in 42 days, while the other recovered after its diet had been changed to rye. Evidently the quantity of bran included in the dietary was not sufficient to afford protection against polyneuritis, since it has been found that rices with more than 50 per cent. of the pericarp removed can produce polyneuritis in fowls, while those with less than this removed, afford complete protection.

Experiment 3.

Two pigeons fed on polished Patna rice (60 g.) plus the white of egg (15 g.) lost weight and died of polyneuritis in 39 and 62 days respectively.

Experiment 4.

Two pigeons fed on polished Siam rice plus a complete salt mixture in solution (per os) lost weight, and died from polyneuritis in 47 and 49 days respectively. Two control pigeons fed on polished Siam rice died in precisely the same time.

Experiment 5.

Two pigeons fed on polished Siam rice plus a plentiful supply of burnt rice polishings lost weight, and one died in 26 days; the other became polyneuritic after 59 days and was then given 5 g. of butter daily in its food. It improved slightly at first, but gradually became weaker and was definitely polyneuritic 4 days later. It was then given some rice polishings moistened in water and it gradually recovered (Plate 10, Figs. 1 and 2).

PLATE 10.

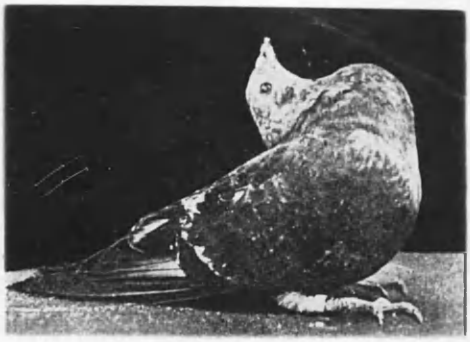


Fig. 1.

Fig. 2.

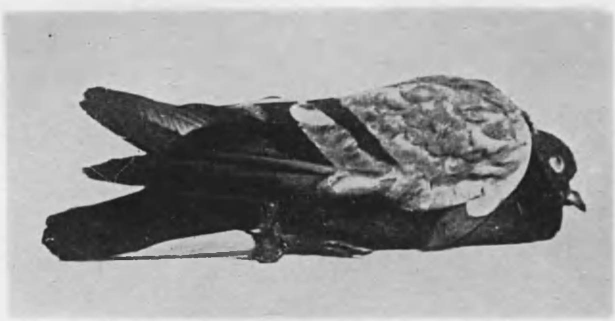


Fig. 3.

Fig. 1. Pigeon suffering from polyneuritis as the result of a diet of polished rice, showing opisthotonos.

Fig. 2. The same pigeon the day after having received a curative dose of rice polishings.

Fig. 3. Pigeon suffering from polyneuritis, showing emprosthotonos.

(ii) Chemical and Biological.

Rangoon, Ballam and 'parboiled' rices were fully examined by the usual method. The purified extracts were toxic while the "glucoside mixtures" were found to be inactive or only very slightly active. Decomposition of the lead acetate precipitates usually yielded a very minute amount of acid substances, which however proved to be toxic when given hypodermically (as neutral bodies) to frogs.

The cold water extract of 10 kilos. of Rangoon rice yielded from the decomposition of the lead acetate precipitate, .4 g. of an alcohol-insoluble acid substance, .05 g. of which (neutralised with sodium bicarbonate) given hypodermically to a frog produced little or no effect even after several days. There was also isolated in the form of acicular crystals a very minute quantity of an alcohol-soluble acid, which X in a dose of .008 g. produced in a frog complete paralysis when given hypodermically as a sodium salt. Marked fibrillary twitchings were present, and at one stage the frog had a tetanic spasm of the hind legs. The paresis lasted for 48 hours, and was followed by increased spinal reflexes lasting for several days. Chemical examination of this acid by micro-methods showed that it was not one of the ordinary plant acids, but the amount of material at the writer's disposal did not permit of a fuller chemical analysis. The yield obtained (.3 g.) was probably only a mere fraction of the total amount present in the rice.

20 kilos. of 'parboiled' rice were then examined by similar methods. The decomposition of the lead acetate precipitate by sulphuretted hydrogen yielded 10 g. of an acid substance, insoluble in strong alcohol which however, was found to be only slightly active when tested on frogs; doses of .05 g. (neutralised) produced only slight increase of reflexes. There was isolated from the alcohol a very minute amount of a crystalline acid which however, had very little action when tested on frogs, and doses of .02 g. produced only a slight increase of reflexes.

Since the process of 'parboiling' is much favoured (by ~~foreigners and~~ public authorities) for the production of a rice, both harmless and possessing marked sustaining qualities, it is not surprising that this sample proved innocuous. The process probably destroys or removes the toxic principle present in the cereal. The writer understands nevertheless, that sporadic cases of beri-beri have arisen from its use. It must always be remembered however, that the water-soluble vitamin B complex is a further complicating factor in this disease.

Lastly the cold water extract of 12 kilos. of Ballam rice was fully examined by the usual method. This is the variety used by lascars on board British sea-going vessels, and the writer understands that it is subjected to a process somewhat similar to 'parboiling'.

Decomposition of the lead acetate precipitate by sulphuretted hydrogen yielded 1.55 g. of an alcohol-insoluble acid substance, which however had little or no effect on a frog, when given hypodermically in a dose of .05 g. (neutralised). On concentrating the alcohol which was used in the precipitation of this material, a minute amount of crystals was obtained. Their crystalline form (rectangular prisms) was identical with that of the corresponding crystals from Rangoon and 'parboiled' rices. The yield obtained however, and the difficulty experienced in separating them from their mother liquor, did not permit of further investigation of their chemistry or pharmacology.

The results obtained with this rice also favours the view that the process of 'parboiling', either removes or destroys the toxic substance present in the cereal.

Common Rye (Secale cereale).

This cereal (Plate 8, Fig. 2) is supposed to be the cultivated form of Secale montanum, a wild perennial species, occurring in the more elevated districts along the shores of the Mediterranean. Unlike wheat, its cultivation does not appear to have been practised at a very early date. Alphonse de Candolle (42), who has collected the evidence on this point, draws attention to the fact that no traces of this cereal have hitherto been found in Egyptian tombs or in the earlier Swiss dwellings. The absence of any name for it in the Semitic, Chinese and Sanskrit languages is also adduced as an indication of its comparatively recent culture. Its cultivation was well known to the Romans in Pliny's time (62), but not to the ancient Greeks, who were used to wheaten bread and objected to the black, malodorous product of Thrace and Macedonia. The writer finds that Theophrastus (43) and Galen (63) are the only Greek writers who mention the rye. It is also mentioned in the English version of the Old Testament (64), although in the opinion of Sprengel (65), spelt wheat (Triticum Spelta) is meant. Rye has always been essentially the bread corn of the Teutons, and it seems likely that it was introduced into Roman Britain by the Germanic invaders. During the Middle Ages, it was fairly extensively cultivated in this country, chiefly as food for the poor, although it seems clear that their bread was never made exclusively from rye. The improvements in agriculture in Britain during the 18th century however, resulted in rye being almost entirely displaced by wheat. In 1925 rye was limited to 50,000 acres in England, and only 30,000 acres were allowed to ripen as forage for horses and cattle, the rest being ploughed in as green manure.

Rye is still the chief cereal in the large belt of Europe, extending from Holland across Northern Germany, Czechoslovakia, Austria, Poland and Central Russia to the Ural Mountains. It is also very extensively cultivated in Sweden, Denmark, Norway and Belgium. The following table shows the production of rye and wheat in these countries for the year 1929.

Relative Importance of Rye and Wheat.

	Rye.	Wheat.	Rye:Wheat Ratio
Poland	246 million bushels	60 million bushels	4.1
Germany	321 " "	123 " "	2.6
Netherlands	13 " "	4.7 " "	2.1
Austria	19 " "	11.6 " "	1.6
Czecho-slovakia	64 " "	48 " "	1.3
Russia	756 " "	783 " "	0.96
Sweden	16 " "	19 " "	0.87
Denmark	10 " "	12 " "	0.87
Norway	0.56 " "	0.73 " "	0.77
Belgium	11 " "	16 " "	0.68

It is evident that Russia is the greatest rye-producing country in the world, allotting 37 per cent. of her total acreage of tillable land to this crop.

In the parts of Europe mentioned, rye forms the chief breadstuff, being used either alone or in combination with wheat in the making of various kinds of 'black bread.' Although this bread lacks the elasticity, friability and vesicularity of wheaten flour bread, it holds an important and favourite place in the dietary of all these peoples. In nutritive value, or measured by the amount of gluten it contains, it stands next to our own white bread, although in its content of the water-soluble vitamin B complex, it is superior to the latter.

The straw of the rye is prized on account of its length in making hats, and in the manufacture of paper, while the bran is used for cattle food.

Nervous Ergotism. Ergot of rye has at most times been incriminated as the cause of convulsive or nervous ergotism, despite the fact that this condition has never been properly demonstrated clinically by the action of the active principles in ergot.

Several outbreaks of this disease, sometimes on a large scale, have occurred as a result of ingestion of rye, which was shown to contain either no ergot, or at most, only a very small amount. In 1846 Vleminckx (66) reported on an outbreak in three Belgian prisons, two years earlier, in which 160 persons were involved; the symptoms were quite typical of convulsive ergotism. Little or no ergot was found in the rye, and this cereal as well as wheat was exculpated by the prison authorities.

In man, the onset and course of the nervous symptoms in convulsive ergotism bear such a close resemblance to those produced in monkeys fed on monotonous cereal diets, and also to those of pellagra, that they suggest very strongly that the cause is fundamentally the same in all, and that the disease is not due to any poisonous substance elaborated by the fungus Claviceps purpurea, but on the contrary, to a toxic principle present in the grain itself. This opinion is strengthened by the records which show that other cereals have produced outbreaks of ergotism. One case occurred in the family of a poor agricultural labourer near Bury St. Edmunds in 1762, and was fully described by Wollaston (67) and Bones (68) in the Proceedings of the Royal Society. There was no rye in the neighbourhood, and apart from dried pease, pickled pork, bread, cheese, milk and small beer, the family lived on bread from "clog-wheat or revets."

In 1931 Professor Mellanby (69b) showed that dogs fed on a diet consisting chiefly of unergotized rye embryo, supplemented with various foodstuffs, developed paralysis of the legs, and after death showed degenerative changes in the spinal cord. He concluded that the embryo contained a nerve toxin, and that infection by ergot resulted in the production of an increased amount of this toxic agent.

The present investigation conducted on monkeys, has shown that a poisonous acid substance exists in the rye grain. Animals fed on rye, or those receiving hypodermic injections of the neutral salt, exhibited many of the salient features of nervous ergotism. As in the case of other cereals, different samples of the grain varied much in their toxicity, one being innocuous or only slightly toxic, while another proved to be very poisonous.

It seems highly probable that rye in the quantity ordinarily consumed, as a supplement to a good mixed dietary, is innocuous but that in times of famine and dear foodstuffs, an excessive use of black bread made from it, gives rise to local outbreaks of poisoning.

Experimental.

(i) Preliminary Feeding Experiments.

A monkey was fed on Scotch rye meal cooked by steaming, with the addition of butter, milk and fruit daily. For 50 days there was little or no change. After 65 days it became decidedly weaker with its arm flexed at the wrist and elbow, and later the paresis spread gradually to its other limbs. After 94 days it was completely paralysed and lay on its side; it exhibited at times coarse muscular twitchings and tremors.

After death, it showed the usual degenerative changes in the nervous system.

(ii) Chemical and Biological.

Two separate lots of rye grain (free from ergot and other grains) were fully investigated, and were found to vary greatly in their poisonous content, one lot being very toxic, while another sample proved to be only slightly so.

The cold water extract of 12 kilos. of rye, grown in Scotland, was submitted to a full examination by the usual method.

The purified extract was toxic while the "glucoside mixture" was only very slightly poisonous and became inactive after continued treatment with lead acetate. Decomposition of the lead acetate precipitate yielded 6·6 g. of an alcohol-insoluble acid substance, which in doses of ·05 g. (neutralised with sodium bicarbonate) produced little or no effect when given hypodermically to a frog. The alcohol-soluble acids were isolated in the form of their sodium salts which crystallized; the total yield was 20 g. Fractional crystallization yielded first di-sodium hydrogen phosphate, and finally the sodium salt (7 g.) of an organic acid. This was found to be very active when given hypodermically to monkeys. A small amount of the free acid was obtained in the form of sheaves of needle-shaped crystals by decomposing its insoluble lead salt by hydrogen sulphide, and allowing the material to crystallize. Despite the difficulty experienced in separating these from their mother liquor, micro-methods of analysis showed the absence of any ordinary plant acid.

A large monkey was given hypodermically 3 g. of the sodium salt of the active acid, and almost immediately became depressed and much weaker than usual. After an hour, it was much worse, and was given hypodermically an additional 2·5 g. The animal immediately became very paretic and lay on its side (Plate 11). There was some slight irritation at the point of injection. Three hours later it was very depressed and moved about with difficulty, its hind legs being very much affected. The following day it was still weak. and depressed generally. It remained in this condition until the third day after the injections, when it seemed to recover.

A rabbit (1750 g.) received 1 g. of the sodium salt of the active acid hypodermically. There was no immediate effect, although the animal was a little quieter than usual, and during the six hours immediately after the injection, it seemed normal. It continued in this condition for several days when observations were stopped. This experiment corroborates the results of other work which has shown the non-poisonous nature of the active substances present in peas

RYE.

PLATE 11.

Monkey after hypodermic injection of sodium salt of active acid.

and cereals when given hypodermically to rabbits. These animals thrive, or are at most, very little affected when fed on monotonous cereal diets over extended periods.

Frogs were completely paralysed by hypodermic injections of ·03 g. of the sodium salt of the active acid. The paralysis usually continued for several days, and where the animals survived, was followed by marked increase of reflexes. Doses of 1 to 3 cg. of the free acid caused similar paralysis or depression, and in some cases the animals exhibited violent tetanic spasms. This was usually followed by increase of spinal reflexes lasting for several days.

The cold water extract of 10 kilos. of English rye was then submitted to a full examination by similar methods.

The purified extract was only slightly toxic while the "glucoside mixture" was completely non-toxic. Examination of the products obtained from the decomposition of the lead acetate precipitate, showed the presence of 3.4 g. of an alcohol-insoluble acid body, and a very minute amount of a crystalline alcohol-soluble acid. Doses of ·05 g. of the alcohol-insoluble substance neutralised with sodium bicarbonate produced in frogs no effect, except a very slight increase of reflexes. The alcohol-soluble acid crystallized from water in sheaves of long slender needles. A very minute dose (less than ·01 g.) proved to be very toxic when given hypodermically as a sodium salt to a frog; it produced paralysis followed by increase of spinal reflexes lasting for several days. The small yield obtained however, did not allow of a more extended chemical examination.

Oats (Avena sativa).

Oats (Plate 8, Fig. 3) are the fruits of a cultivated grass, which according to Alphonse de Candolle (42) was not cultivated by the Hebrews, Egyptians, ancient Greeks or Romans. Central Europe appears to be one of the localities where it was first grown, since its grains have been found amongst the remains of the Swiss lake-dwellings (Bronze Age). Pliny (62) alludes to bread made of it by the ancient Germans, while Galen (63) observes that it was abundant in Asia Minor, where it was made into bread as well as given to horses. It is also mentioned by Hippocrates (70), Theophrastus (43) and Dioscorides (71) under the name βρόμος.

It is now grown extensively in all parts of the globe, and statistics for the whole world, exclusive of Russia and China, show a total annual production of 57,000,000 tons. People are beginning to appreciate more the value of oatmeal as a human food, and to recognize that so far as chemical composition and the ratio of the nutrients are concerned, it is an almost perfectly adjusted food. According to some writers, it is supposed to cause heat in the stomach and various cutaneous affections, but the age-long experience of mankind all over the world conclusively demonstrates the harmlessness of this meal when taken as part of a good mixed dietary.

Gross over-consumption however, especially if combined with a very limited supply of other foodstuffs, is a very different matter and that this, short of causing a disease might quite conceivably give rise to some deterioration of health, is borne out by experimental and clinical evidence.

In 1894 Weiske (52) recorded that the continued feeding of rabbits with oats alone, caused a diminution of the skeleton, more especially of the inorganic content. He concluded that the effects were due, not to insufficient lime in the food, but chiefly to its acid nature. Scheunert, Schottke and Loetsch (72) showed that horses fed on oats chiefly, developed osteomalacia, while the writer has heard of cases where sheep and horses fed mainly on oats, died from gradually deepening paralysis. Morgen and Beger (73), and later Funk (49b), pointed out that rabbits fed on oats developed acidosis which could be prevented or cured by the addition of sodium bicarbonate to the food. In this connection, it is interesting to observe that other writers have noticed that animals fed chiefly on oats are much less resistant to acid poisoning (for example tartaric acid) than those receiving other foodstuffs.

In 1925 Professor Mellanby (69a) showed that a diet containing a preponderance of oatmeal, especially in the absence of an adequate supply of vitamin D, was capable of interfering with bone-calcification in dogs. He was unable to explain the method whereby oatmeal brought about this rickets-producing effect. In 1931 he further showed (69b) that this cereal, bulking largely in the diet of a dog, was capable of producing certain degenerative changes in the spinal cord, which could be prevented by the inclusion of an adequate supply of vitamin A in the dietary.

On the clinical side Sambon and Chalmers (74) have recorded a case in which a woman (aged 40), who since youth had been in the habit of eating raw oatmeal, developed pellagra and died of insanity.

The present investigation, however, has shown that the explanation of these cases lies in the presence of an acid substance or substances having an action similar to those found in other cereals, and which can be extracted by similar chemical methods.

Experimental.

(i) Preliminary Feeding Experiments.

A monkey fed on a diet of Scotch oats porridge with the addition of butter, milk and fruit daily, became definitely paretic after 66 days; its hands were weak and flexed, its knees and back bent, but at times it was fairly active when roused from the somnolent attitude it inclined to adopt.

Rabbits and guinea-pigs fed on a diet of oatmeal cooked by steaming, with the addition of cabbage (ad libitum), increased in weight and seemed to thrive, but when the cabbage was discontinued they became weak, lost weight and died in 12-13 and 10-12 weeks respectively. The addition of orange juice to their dietary did not have any beneficial effect. They all lost much hair and were in a miserable condition for several weeks preceding death.

(ii) Chemical and Biological.

The aqueous extracts of several samples of oatmeal were examined by the usual lead method and were found to vary considerably in their content of the toxic substance.

The purified extracts proved to be toxic while the "glucoside mixtures" were only slightly so or not at all.

Decomposition of the lead acetate precipitate by sulphuretted hydrogen yielded a crude physiologically active principle, insoluble in strong alcohol. This proved to be a white, amorphous, hygroscopic substance, and gave the same precipitation reactions as the corresponding substance from the maize cake. It was not further purified, and the following experiments were conducted with it on monkeys and frogs by Professor Stockman.

Monkeys.

A Rhesus monkey which had been fed for 58 days on a supplemented diet of Scotch oats porridge, without showing any marked symptoms except loss of agility, was given 0.25 g.

of the crude principle (neutralised with sodium bicarbonate). In a few minutes it became very paralysed and lay down on its side. The paresis continued for several days with flexion of the joints and marked muscular tremors, and a necrosis formed at the site of injection. About one month later, the same animal was given 1 g. of the crude active principle (neutralised with sodium bicarbonate) hypodermically, and in a few minutes became very helpless and paretic. The paresis, with flexion of the joints, continued for 4 days when it was killed by chloroform. After death it showed the usual degenerative changes in the nervous system, as shown by Marchi's method of staining.

Another monkey which received hypodermically 1.4 g. of the crude principle (neutralised with sodium bicarbonate) showed similar paralysis which lasted for several days. There was also marked irritation at the site of injection and after death it showed similar degenerative changes.

<u>Frogs.</u>

When doses of ·04 g. of the crude principle (neutralised with sodium bicarbonate) were given hypodermically to frogs, they produced paralysis for several hours followed by increased spinal reflexes lasting for several days. Larger doses produced death in a few minutes, while smaller doses resulted in depression followed by increase of reflexes lasting for some days.

Wheat (Triticum vulgare).

Wheat (Plate 8, Fig. 4) is the grain of a cultivated grass which according to De Candolle (42) has been grown by man since the most remote times. Its cultivation is older than the most ancient languages, each of which has independent and definite names for it, such as the Chinese mai, the Hebrew chittah and the Sanskrit names godhuma, gahu or gahung. According to De Candolle, it originated in Mesopotamia whence it spread to all parts of the world where the climate and soil were suitable.

In the earlier ages, wheat was an esteemed article of food (Leviticus ch. ii) and is frequently mentioned by Hippocrates (70) and Pliny (62). Since its introduction into Britain by the Romans, it has been grown on a fairly large scale especially on the East coast. Nevertheless Great Britain has always been dependent on foreign countries and her Empire for the greater part of her requirements. Statistics for 1922-26 show an average annual production of 1,300,000 tons, while during the same period 5,074,000 tons were imported annually, chiefly from Canada and the United States of America.

Since wheat is the most esteemed of all cereals, particularly for the making of bread, the increase in its cultivation and use has marked the progress of agriculture and wealth in many countries, particularly in Great Britain.

From its almost universal use in bread-making in Europe, it has now come to be known as "the staff of life." The chemical composition of the grain shows how admirably it is suited to this purpose.

<u>Composition of Wheat Grain</u> (Indian).

Water	12.5 per cent.
Proteins	13.5 " "
Starch	68.4 " "
Oil	1.2 " "
Fibre	2.7 " "
Ash	1.7 " "

Despite its admirable qualities however, the use of wheat alone as a foodstuff is unable to maintain life indefinitely. In 1916, McCollum, Simmonds and Pitz (75) and Hart, Miller and McCollum (76) investigated the nature of these deficiencies in the wheat embryo and grain respectively. They found that pigs fed chiefly on wheat germ or grain for several months, even in the presence of all the recognized factors for growth, showed finally a considerable loss of weight and marked paralysis of the legs. After death, degenerative changes were found in the spinal cord. The authors attributed their results partly to the inherent toxicity of the wheat, and partly to a deficient supply of inorganic salts. In 1931 Professor Mellanby (69b) showed that dogs fed chiefly on wheat germ supplemented with all the recognized factors for growth, developed weakness and later, paralysis in the legs. After death, there was much degeneration in the spinal cord as shown by the Marchi method of staining. He also stated that this cord degeneration could be prevented by including in the dietary, an adequate quantity of mammalian liver oil, as a source of vitamin A.

The present investigation which was conducted on a sample of wheat grown in Scotland, showed the presence of an acid, whose sodium salt on hypodermic injection proved to be very toxic to monkeys, rabbits and frogs.

Experimental.

Preliminary feeding experiments showed that a monkey fed chiefly on wheat (cooked by steaming), supplemented with butter, milk and fruit, gradually became paretic. Another monkey died 6 hours after it had received per os, a cold water extract of 400 g. of ground wheat. This was evidently a case of acute poisoning.

These experiments undoubtedly disproved any suggestion of deficiency in the grain, and showed that this sample of wheat contained some principle which was poisonous to monkeys.

Chemical and Biological.

The investigation was conducted on the cold water extract of 15 kilos. of wheat grown in this country.

The purified extract was toxic, while the "glucoside mixture" was found to be inactive. Decomposition of the lead acetate precipitate yielded 6.2 g. of an alcohol-insoluble acid substance, which in a dose of ·05 g. (neutralised with sodium bicarbonate) failed to have any marked action on a frog when administered hypodermically or per os.

The alcohol-soluble acids from the lead acetate precipitate were isolated in the form of their sodium salts (18 g.). These were fractionally crystallized from water, the first two crops of crystals consisting chiefly of sodium phosphate. The third crop (5.5 g.) was almost entirely free from phosphate and consisted of the sodium salt of a toxic acid. The amount of material at the candidate's disposal did not allow of a complete chemical examination, but by adopting the standard micro-methods of analysis, the absence of the commoner plant acids was demonstrated. The following biological experiments showed the active nature of the material when given hypodermically to animals.

A small monkey was given hypodermically 2 g. of the sodium salt of the active acid. In 3 minutes it became weak and lay down on its abdomen at intervals. After one hour it became very paretic and either sat with its back bent, supporting itself against the wall, or lay down on its stomach (Plate 12). Its condition remained unchanged

WHEAT.

PLATE 12.

Monkey after hypodermic injection of 2 g. of sodium salt of active acid.

throughout the rest of the day, and in addition, it exhibited flexion of the fingers and wrists which prevented it from walking normally. Next day it was still weak but had recovered slightly, and on the third day it seemed normal again.

A rabbit (1155 g.) received hypodermically 1 g. of the sodium salt of the active acid. In 15 minutes it exhibited a tonic spasm of the hind legs, and for the rest of the day it remained very weak and depressed. The following day it seemed normal again.

2 to 4 cg. of the sodium salt given hypodermically to frogs produced paralysis of the brain and cord, followed by increased reflexes for several days where the animals survived.

Samples of the commercial products (viz. germ, flour and bran) of the constituent parts of the wheat grain were also examined by the same methods and were found to contain an active acid substance insoluble in strong alcohol. The yield from the different parts varied, being greatest from the embryo and least from the flour. In its action on frogs and in its chemical reactions it resembled the corresponding substance obtained from the maize cake. The following table shows the relative yield and action of this substance from the individual parts of the wheat grain.

WHEAT.

Acid Principle insoluble in Alcohol (from decomposition of lead acetate precipitate).

Part of grain used.	Yield per kilo.	Action of ·05 g. (neutralised) hypodermically to frog.
Whole grain	·46 g.	Slight action followed by increase of reflexes.
Endosperm (flour)	·09 g.	Nil.
Germ	·9 g.	Produced paralysis for 24 hours, followed by reflex increase.
Husk (bran)	·3 g.	Slight action followed by increase of reflexes.

CONCLUSION.

This investigation, which was conducted in collaboration with other workers, has shown for the first time the presence of toxic substances in the commoner pulses and cereals. The remarkable results obtained must of necessity throw some light on the aetiology of the diseases lathyrism, nervous ergotism, beri-beri and pellagra, and also on the much larger problems of human nutrition in general.

It has been demonstrated that the active principles of maize, rice, rye, as well as lathyrus and other leguminous seeds give the same chemical reactions, have an action on animals which is always qualitatively the same and produce identical pathological changes in the nervous system when administered orally or hypodermically. The idea at once suggests itself that the conditions resulting from the excessive use of these foods are only different manifestations of a disease, which however arise from the same fundamental cause. This view is further strengthened by the occurrence of identical pathological changes in the nervous system in these conditions. The research has further shown that the production of this syndrome is not solely a question of vitamins since all the experimental animals received vitamin-containing foods in abundance. The precise mode of action of the active principles is not yet known. However it is probable that they are not only irritant poisons in themselves, but that they have a disturbing effect on the mineral metabolism of the body.

The new field which has been opened out is a large one and one which requires much further study. A method of isolating the crude active principles has been found, and it only remains for other workers to manufacture large quantities of these for the further investigation of their chemistry and pharmacology. The knowledge thus derived would undoubtedly strengthen the art of medicine, whether applied to the prevention or the cure of the aforementioned diseases.

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