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Biochemical remarks in connection with the lathyrism problem*

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Introduction

Lathyrism is the name given to a chronic disease, the etiology of which is still unknown, but which is unanimously attributed to the poisoning that consumption of the blue vetch *(Lathyrus sativus)* produces if used as the basic constituent of the food ration.

Although the blue vetch is widely used for food, cases of lathyrism are not very frequent and they manifest themselves generally in the form of local outbreaks, and in periods and regions where, owing to circunstances, a food shortage or an anomaly of living conditions, force people to consume it on a large scale or to live on it as practically the only food.

During the recent world war a considerable number of cases of lathyrism were recorded in Spain. Nearly all of them occurred in the poorest central provinces, for which reason investigation into the possible causes of the outbreak of the disease has been carried out in various directions.

In the present paper it is studied the biochemical composition of the seed to the ingestion of which this illness must be attributed; furthermore some of the possible toxic factors are taken into consideration, as several investigators attribute the outbreak of the disease to them.

Experimental

I. Global composition

Firstly the basic constituents were determined on samples of seeds from five different places, in all of which numerous

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cases of lathyrism were recorded. The figures presented in Table I correspond to the average value of two determinations of each of the samples.

TABLE I

Origin	Water per cent.	Glucids per cent.	Lipids per cent.	Protids. per cent.	Ashes per cent.
Valladolid.	11'30	58'68	1'09	20'91	—
Palencia	12'80	59'15	1'05	20'36	2'83
Guadalajara	—	—	1'09	24'33	2'58
Cuenca.	11'04	58'37	1'21	24'42	2'63
Madrid.	10'82	—	1'12	27'48	—
Average figures:	11'48	58'37	1'11	23'50	2'68

The methods were the usual ones. The glucides here indicated correspond to what is commonly classified as *extractive watersoluble non-nitrogenous substances.* The protid number is obtained by multiplying by the percentage of total nitrogen (Kjeldahl, catalyst: selenium).

II. Glucids

The glucidic components were divided in fractions of the following groups: a) monoses, b) holosides. c) starch, d) cellulose, e) pentosans. f) phytins, g) lignins. The figure corresponding to the total of glucides, after previous inversion (h), was also determined.

a) and b) were titrated as usual (Met. of anal. Ass. agr. Chem.). c) according to Lintner and Belschner, d) by the method of Scharrer and Kurshner, e) according to Tollens and Kruger's method (1896), titrating the furfural by precipitation with phloroglucinol, deducing from the weight of the precipitated phloroglucid the quantity of pentosans, according to the formulae given by Krober (1900).

For the titration of (f), Heubner and Stadler's technique (1914) as described by Averil and King (1926).. was emploved, introducing the modification of Andrews and Baley (1932) in orden to avoid the inconveniences of the final titration.

g) Titration according to Ost and Wilkening (1910).

The corresponding results are summarized in Table II.

TABLE II

	Per cent.
Monoses	0
Holosides	1·53
Starch	34·84
Cellulose	6·16
Pentosans	6·80
Phytin	0·46
Lignin	1·52
Total glucids	56·98

III. Lipids

The physico-chemical constants which were determined in oil were the following: characters of paralyzation of organs: the oil obtained by extraction, is a viscous liquid which gets turbid on cooling, has a strong pungent smell and a chracteristic taste, its colour is very pronounced and for this reason it can be read directly from the tintometer. Dissolved in ether at a concentration of 10 % (v/v) it gives the following reading in the Lovibond tintometer:

TABLE III

Lovibond units:				Developed colour	
Red	Yellow	Blue	Neuter tint	Predominant colour	Componcnt colour
3·9	30	1·9	1·09	2	2·61
				greenish yellow	orange yellow

The specific weight at 20° was D = 0.9285

Refraction index (Abbe refractomer) at 20°C 1.4768
at 40°C 1.4681
refractometric degree = 70 at 35°C

Rotatory power $[\alpha]_D^{20°} = 0°$ (in benzenic solution at 5 %).

Acidity index: 44.99

Acidity expressed in oleic: 22,6 per cent.
Acidity expressed in sulphurous anhydride: 3,2 per cent.

Index of saponification: (Kottstorfer) . .	=	172
Index of iodine (Rosemund-Kuhhenm) .	=	98
Index of Henner	=	84·05
Index of Reichert-Meissel	=	1·992
Index of Acetyl	=	30·45 per cent
Index of insaponificable residue. . . .	=	16·20 per cent (in weight)
Index of ester	=	127·01
Glycerin.	=	6·94 per cent (in weight)
Lecithins (Kunze and Bauer, 1941) . .	=	0·48 per cent (in weight)

Fatty acids: Physico-chemical constants:

Refraction Index	=	1·4590
Solidification point	=	22·23°C.
Melting point	=	26°C.
Index of neutralization	=	79
Index of iodine	=	108·18

Insaponificable residue: physico-chemical chracteristics: solid at ordinary temperature, of brownish red colour and pungent smell.

Refraction Index: at 40°C . . .	=	1·512
Iodine Index	=	95·42
Esterols (digitonin)	=	36·2 per cent.

IV. Protids

General determinations:

Total nitrogen: (Kjeldahl) 4,27 per cent.

Amino-nitrogen: (van Slyke) 1.93 c.c. of. N per cent of flour expressed in alanine: 1.57 per cent.

Nitrogenous fractions:

a) Albumins and amino-acids: (watersoluble fraction): 6.82 per cent. (N Kjeldahl = 1.197 gr. per cent) (fraction soluble in 10 per cent ClNa).
b) Globulins: 12.61 per cent. (N Kjeldahl = 2.23 gr. per cent).
c) prolamins: (fraction soluble in 70 % alcohol) 1.28 per cent. (N Kjeldahl = 0.212 gr. per cent).
d) glutenins: (residual fraction) 6.32 per cent. (N Kjeldahl = 1.11 gr. per cent).

Essential aminoacids:

The total protids were separated and purified by Lugg's method (1938). For the titration of the various amino-acids three hydrolyzed types (hydrochloric according to Tristam (1946), sulphuric (Block 1940) and alcaline) were prepared.

With the alkaline hydrolized type the quantitative study of the amino-acids was carried out by means of the chromatopraphic method with filter papel of Martin and Synge (1941), which enabled the presence of all indispensable components to be proved. Whatman Paper No 4 and various solvents were employed (phenol-cupron 0,1 per cent, collidine, butanol, o-creosol, etc.).

Quantitatively the following amino-acids were determined: Arginine, histidine and lysine according to Block and Bolling (1940). Phenylalanine according to Kapeller-Adler, modification of Kuhn and Desnuelle (1937). Tyrosine and tryptophane according to the adaptation of Block and Bolling of Millon-Lugg's method (1938). Methionine according to McCarthy and Sullivan (1941). Cystine and cysteine by the method of Winterstein-Follin, according to the adaptation of Block. Leucine, isoleucine and valine, according to the technique of Block and Kondritzer (1940), adaptation of the method of differential microoxidation of Fromageot-Heitz (1939), determining the resulting acetone of the oxidation of leucine and valine by the gravimetric technique of Cook and Smith (1930) and the methylethyl cetone which corresponds to leucine by colorimetry of the product of reaction with salicylic aldehyde according to Fabinyi (1900). Threonine and serine by the method of oxidation with periodate of Shinn and Nicolet (1943). Proline, by precipitation with Reinecke salt, according to Miller (1936).

The analytical results are shown in Table IV.

TABLE IV

Amino-acids	Per cent in total protids of blue vetch
Histidine	4'1
Arginine	3'2
Lysine	1'6
Phenylalanine	7'9
Tyrosine	6'0
Tryptophane	1'0
Methionine	0'6
Cystine and Cysteine	1'0
Valine	6'2
Leucine	7'1
Isoleucine	1'9
Threonine	3'3
Serine	4'2
Proline	5'8

V. Mineral constituents

a) Composition of ash:

Chlorides (expressed in NaCl on the total ash). .	3'28 grs. per cent.
Sulphates (expressed in So_3)	4'25 » » »
Fhosphates (expressed in P_2O_5).	20'80 » » »
Calcium (expressed in CaO)	12'62 » » »
Magnesium (expressed in MgO)	3'87 » » »
Potassium (expressed in K_2O)	47'60 » » »
Sodium (expressed in Na_2O)	20'35 » » »

b) Trace elements:

Spectrochemical determination showed the following results, in percent (of the ashes): aluminium, 0.1; boron, traces; copper 1; iron, 1; lithium, traces; manganese, 1; molybdenum, 0,5; nickel, 0,001; lead, 0,0001; titanium, 0,001; fluorine, traces.

c) Investigation of selenium:

The possibility that lathyrism may be caused by massive poisoning with selenium (Fernández and López Bustos, 1946) has already been mentioned, and as selenium easily volatilizes its determination in the ash is not possible. Thus it was studied by means of microchemical techniques in the product resulting from the destruction by oxidation of the organic matter of the flour, by a mixture of perchloric, nitric and sulphuric acids. Eight different samples of blue vetch gave negative results, being studied by the micro reactions described by Feigl (1935), 1) with thiourea, 2) with iodhydric acid and 3) with pyrrole.

VI. Vitamins

Carotenes: separated by the method of Wolff (1938), they were titrated according to Kroguis (1936) by colorimetry. Thiamine was tritrated according to Crisner and Deltomee (1934) using the modification of Santos-Ruiz and Torres (1943). Riboflavine was determined according to Emmerie, employing the Pulfrich photometer. Nicotic acid (vitamin PP), was studied according to Melnick and Field (1939), modification of Montañés (1942). Vitamins C, D and E were also studied, but gave negative results.

The results obtained for the content of vitamins of the flour of blue vetch, in micrograms per cent were: A, 397; B_1, 182; B_2, 267, and nicotinic acid, 7.500.

VII. Alcaloid

With scope of studying the biological activity of the alkaloid that after Mayer, Stossmann and Dilling and Pierre Marie is found in this seed, we employed to its extraction the following technic that was suggested by Santos-Ruiz and Portús-Serrano (1946).

We worked with ten kilograms of previous ly pulverized blue vetch; the product was macerated with a dilute solution of tartaric acid, and afterwards it was filtered and defecated; filtering once more and submiting it to a current of sulphydric acid to detach the excess of lead; filtering and concentrating the resultant liquid does not give the alkaloid reaction. The so voluminous precipitates that are formed by defecating and by the current of sulphydric acid are undoubtedly the cause of so great phenomenons of superficies that they retain or keeps back the alkaloid in case of existing.

The general techniques of alkaloids extraction also fails because as soon as the product is rendered alkaline, the alkaloid volatizes itself.

Therefore we change the technic and after several experiments we were able to obtain a method in which it is not necessary to defecate with lead subacetate; by this new means is avoided the adsorption of the following precipitates: lead tartrate, proteinic precipitates and lead sulphur.

The technique was as follow:

Ten kilograms of blue vetch seed previously pulverized are put in maceration with a diluted solution of nitric acid during twenty four hours. The maceration is performed by fractionation, placing in large precipitate vases of 4 litres capaticy 1½ kilogram of blue vetch flour, adding afterwards 50 ml. of concentrated nitric acid previously disolved in 1½ liters of water. After 24 hours of frequently shaking the reaction of the macerate is acid. Rejoining the macerates, they are squeezed and the resultant liquid filtered and concentrated. In proportion as the concentration of the liquid increases the nitric concentrates itself and the albumins are then precipited; in consequence we made a series of subsequent concentrations and filtrations till the albumins reactions with concentrated nitric, sulfosalicilic, trichloroacetic, and methaphosphoric acids were negative.

The concentrated liquid already without proteins yields strongly positive the Bourchardat and Mayer's alkaloid reactions and precipitate with the picric acid.

To prove the biological toxicity or activity of the concentrated, we were making the following experiences:

1st. — It was prepared a concentrate of alkaloid parting of the previously obtained one, and the pH of which was 1, according it to a new pH of 6 for which we neutralize with alkali (sodium hydroxide) controlling with Merck indicator paper.

2nd. — We prepare an aqueous solution of nitric acid to PH 1, and accorde it to a pH of 6, adding sodium hidroxide in aqueous solution. Injecting a lot of three rats, two of them with the 1st., concentrated of alkaloid and the third one used as control with the 2nd. solution. The injectios were applied by subcutaneous may, in alternate days and of 2 c. c. in quantity. On the third injection the rats injected with the alkaloid concentrate showed a necrosis of skin at the affected zones, but neither died not experimented symptons of lathyrism and its growing was normal. After a period that oscillates between 30 to 40 days without injecting them, the necrosis commences to disappear and the skin and hair regenerate themselves. The rat control has experimented no anormality.

It must bear in mind that the liquid problem is quickly passing of pH 6, to the most absolute neutrality, and in these conditions continues giving the alkaloid strongly positives reactions.

It was performed the identification of selenium in the concentrated of alkaloid, employing the reaction that are pointed in former pages of the iodhydric acid and pyrrol, both with negative issues.

VIII. Saponins

The investigation of saponins by the methods described by Villar-Palasí (1946) was negative in any case.

Discussion

The clinical investigations carried out during recent yers, especially those of the school of Jiménez Díaz, have served to centre the problem of the lathyrism, putting aside many hypotheses previously formed as they have been proved not to have sufficient foundation. At present the idea of an infection (virus?), which had been mantained rather a long time, is no longer supported. Among the possible toxic causes the presence of saponines cannot be the origin of this disease either, as they could not be found in the seed.

Thus the discussion on the possible toxic effects of different kind is left open, and also on the much debated question of quantitative deficiency of lathyrism for nutrition. In relation to the nosology of the disease, the terms of reference now

firmly established, are the conclusions of Jiménez Díaz and coll. (1943), published in a paper summarizing their investigations on the pathogeny of lathyrism. They read as follows:

1) Lathyrism only appears where people consume blue vetch.

2) The disease is not produced by eating the blue vetch but by the fact that it is eaten in large quantities.

3) Lathyrism is only produced when, simultaneously to the former condition, the nutrition is too unvaried, i. e. when it is monotonous.

4) Massive ingestion gives rise to lathyrism, even if there is a certain variety in nutrition. Few such cases have been recorded.

5) The lathyrogenic action of the blue vetch is not accidental, on account of mixtures, parasitation, infection or transformation. The appearance of lathyrism depends on the degree of the extension of the ingestion and on the composition of the other food.

6) Evidently factors of predisposition exist, including age and sex, apart from other lesser known ones; from all of them it appears that only 36 % of the individuals submitted to the same nutrition were affected by lathyrism.

7) Lathyrism of echinus is the only known form of this disease, apart from the human illness, but there exist similar effects produced in other animals by ingestion of various legumes.

From the former conclusions the connection of the idea of deficiency with that of the toxic effects can easily be seen, as both of them meet in the etiology of the illness.

Considering the importance of the kind of nutrition with regard to lathyrism concerning different essential food factors, the pronounced deficiency of some indispensable amino-acids is obvious at the first sight. Those of an inferior to the normal porcentaje are: methionine, lysine and isoleucine. Of these methionine is of more importance tran the others, its absence is notable and now more metabolic importance is given to it than bfeore. If we consider its rôle as a protector of the liver against poisoning, we become aware of the importance of its lack in larhyric nutrition, even if the presence of a toxic agent in it is not admitted. The organic scarcety of metionine would weaken the organic defence against the poisoning factor, which may be of little importance under different circunstances.

Ortiz de Landázuri (1944) on studying the factors that produce lathyrism, point out that the knowledge of protecting factors against one-sided vegetatian food, is very old and its application goes back to the most primitive people. Examples are known from isles of the Pacific, where the nutrition is frequently very monotonous and based on the seeds of legumes. These people, as Veen reports, add to their usual food supplements with already traditional names. The "Nuoc Man" e. g.

is a liquid product obtained by destruction by means of certain bacteria of salted fish in the composition of which many methyl radicals are contained, according to the studies of the same author.

It must be pointed out that the geatest importance in the metabolism of methionine lies in the fact that it is a methylating interorganic agent. Substances of such a vital importance as creatine and choline are in their synthesis dependent on methionine, which has to give them the necessary labile methyl groups.

The higher the muscular activity, the greater the formation, consumption and subsequent excretion of creatine, which is an absolute necessary. With greater activity, the consumption and therefore the necessity of methionine increases and in any case the available quantity is preferably used for the formation of creatine (Tidwell, 1946), with priority over any other employment. Consequently the possibility of poisoning increases for individuals of a good development and great activity, circunstances that generally coincide in lathyric individuals. On the other hand for some of the muscular disorders, the synthesis of creatine may be responsible. At present methionine is considered to be an important factor of muscular dystrophy.

In relation to this the experimental creatinuria which is accompanied by thinness must be pointed out. This has been observed in rabbits exclusively fed on blue vetch (Jiménez Díaz and Vivanco, 1942) during the first days and its coincidence with lack of methionine (Tidwell 1946) is of particular significance.

With regard to choline, its intraorganic synthesis (apart from that introduced during nutrition) is equally dependent on the possibility of methylation. In the lipotropic action on the liver, choline and methionine act parallelly, and they also supplement each other in different aspects. The importance of choline and acetycholine in the conduction of the nervous impulse makes these correlations more significant.

The protection against lathyrism given by the proteins of animal origin could be sufficiently explained by its high content of methionine and lysine. The slight improvement made by lathyrism patients on change of diet, could be explained according to Edsall and Anson —apart form the probable irreversibility if its nervous lesions—, by the experimental fact of the temporary loss of capacity to absorb methionine which certain patients show, sometimes for long periods, as a result of earlier deficient nutrition. Under these conditions

the determination of methionine eliminated by the urine is more efficient than the nitrogen balance.

In relation to the toxic factor, an active element in the genesis of lathyric affection it is not very likely know; perhaps we have here a simple ingestion of blue vetch accidentally containing selenium. Certainly the characteristics of poisoning by selenium bear little resemblance to lathyrism; on the other hand the selenium could not be found by any procedure, in the sedd; nevertheless the observation of Fels and Cheldelin (1948) with regard to the desintoxicating action of methionine against selenium is interesting.

As to other possible deficiencies of food diest of lathyric patients, the most probable one to be taken into consideration is that of hypovitaminosis B_1. Although its titration shows that it is present in quantities only slightly less than the necessary minimum, it must be borne in mind that the necessity increases with the proportion of carbohydrates which generally are consumed in large quantities by lathyric patients. On the other hand the symptomatology of the disease favours the opinion of a possible influence of this deficit as a factor that might explain the localization of the lesions.

Conclusions

1st. — In connection with the chief components (protids, glucids and lipids) the seed of the blue vetch offers a high proportion of protids, the biological value of which is however, enough low.

2nd. — In the proteins of the seed occur all the essential aminoacids. It is noted the deficiency in relation with the smallest needs of the human being in *methionine*. It is relatively low the proportion of lysine and isoleucine.

3rd. — The proportion of oligoelements is correct. Among the vitamins that may be of some signification at the clinical picture, there are wanting almost totally the E vitamin, and the presence of B_1 vitamin is deficient.

4th. — On biological proofs it has not been possible to verify the supposed importance that may have in relation with the appearance of the illness, the presence in scarce proportion of an alkaloid isolated but not yet identified.

5th. — The presence of seleninum in the seed, that has pointed as a possible ethiological factor of the lathyrism, has been unable of being comprobated at the analyzed samples. And the same results were obtained in relation with the hypothetic presence of saponins.

Summary

As lathyrism is one of the most typical chronic diseases produced by one-sided deficiency of nutrition, it seemed of the greatest interest to determine the basis of its etiology and treatment with regard to the chemical constitution of the *Lathyrus sativus* seeds, which are the only ones that produce these effects.

In a series of samples of different origins, the proportion of the following constituents was determined:

a) *Glucids*: holosides, heterosides (glucoside: "vicine"), starch, cellulose, pentosans, phytin, lignin, and total glucides, after previous inversion.

b) *Lipids*: Colour, specific weight, refraction index, rotatory power, acidity index, and that of saponification, of iodine, of Henner, of Reichert-Meissel, of acetyl and insaponificable residue, glycerine, lecithin, fatty acids, sterols, etc.

c) *Potrids*: protidical fractions, essential amino-acids.

d) *Vitamins*: A, B_1, B_2, C, D, E. PP.

e) *Ashes*: principal anions and cations, trace elements.

f) Other significant substances: alcaloids, saponins, etc.

On the basis of these determinations some considerations with regard to the importance of the different factors are established, to which is given signification in relation to the appearance of the illness.

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