XXIX. THE RESERVE POLYSACCHARIDE OF THE SEEDS OF FENUGREEK: ITS DIGESTIBILITY AND ITS FATE DURING GERMINATION.

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INTRODUCTORY.

THE seeds of fenugreek (*Trigonella Foenum-Graecum* L.) have been employed, for many centuries, as a drug in Southern Europe, Northern Africa, Asia Minor and India. They are extensively used by many people in Egypt, not only as a food but also as a medicine for different ailments which appear to have no relationship to one another.

The use of the seeds as a food can be seen among the field labourers who constitute the major part of the population and live almost entirely on maize bread to which a small percentage of the ground fenugreek seeds is added. They are also utilised as a food by women in order to give plumpness to their forms.

For medicinal purposes, the seeds are employed as a stomachic, also as a laxative in the form of powder or a decoction. They are given to women after parturition in the form of a decoction sweetened with sugar and form one of the chief ingredients of pills or powders which are administered for the treatment of diabetes mellitus. For some of the purposes mentioned the seeds are used in the germinated form.

Apart from their nutritive value and bitterness which may account for their stomachic properties, they are not known to contain any active principle of therapeutic value. On the other hand, it is known that mucilages act as emollients provided they are not attacked in the alimentary canal and since fenugreek seeds contain a high percentage of a polysaccharide, highly mucilaginous in character, this may explain their use as a laxative. It may also account for their employment in anti-diabetic preparations, since diabetics generally suffer from constipation and any improvement which follows their use may be indirectly brought about by relieving this symptom. The mucilage, as will be seen later, does not influence the blood-sugar, and the seeds may therefore act as a safe nutrient in cases of diabetes.

These considerations suggested the importance of examining the constitution of the polysaccharide, its digestibility and effect on the blood-sugar when

ingested, also its fate in the germinated seeds, since the latter are often employed in this form. It would seem that the polysaccharide is the element in the seeds which is responsible for the alleviation of symptoms in certain medical conditions.

EXPERIMENTAL.

Preparation and properties of the polysaccharide.

For the preparation of this product the bran is employed, since in the milling of the seeds a considerable proportion of the hard, glassy endosperm containing the mucilage remains adherent to the outer spermoderm.

20 g. bran are mixed with about 4 litres of water in a 5-litre bottle, the mixture is shaken in a mechanical shaker for 5 hours and the contents are filtered. Filtration is generally tedious because of the viscid nature of the solution, but it can to some extent be hastened by filtration at the pump, first through calico and then through three folds of filter-paper. Alcohol up to 30 % is then added to the filtrate. A white hair-like precipitate is formed which is transferred to a suitable calico bag through which as much as possible of the fluid is squeezed out. The precipitate is dissolved in water and reprecipitated by alcohol at least three times, and the polysaccharide is finally treated with alcohol of gradually increasing concentration, followed by ether, and placed in a vacuum desiccator over calcium chloride.

The polysaccharide thus obtained is a light white solid which has no characteristic taste or smell and contains 0.83 % of ash. On drying it partly tends to form small masses which powder with difficulty. On redissolving the dry substance in water some of it passes easily into solution while the rest, which is imperfectly soluble even in boiling water, remains in solid suspension in the colloidal solution of the former and for this reason it is difficult to obtain a homogeneous solution from the dry prepared product. The moist freshly precipitated polysaccharide mixes fairly well with different proportions of cold water and it is thus possible to make it into a thick jelly with this solvent. The solutions are highly opalescent. The polysaccharide does not reduce Fehling's solution and gives no colour with iodine even after treatment with sulphuric acid. It is precipitated by basic lead acetate and also by Fehling's solution. It is soluble in hot potassium hydroxide.

The chemical composition of the polysaccharide.

The polysaccharide was hydrolysed with dilute sulphuric acid, the hydrolysate was neutralised with barium carbonate, and the filtrate was decolorised.

On treating the colourless filtrate with phenylhydrazine and acetic acid in the cold a white crystalline precipitate was obtained which was identified, by its crystalline form and its melting-point, as mannosephenylhydrazone.

On heating the mixture of phenylhydrazine and the hydrolysate and examining the osazones formed, only two could be found, one of which was

identical with glucosazone and the other with galactosazone. No osazones of the pentoses could be identified, and the absence of the latter was further confirmed by applying other chemical tests for these bodies.

That the glucosazone obtained was entirely due to the mannose, and not to the possible presence of fructose or glucose as well, was shown by applying the chemical tests for fructose to the hydrolysate which gave negative results. Glucose was proved to be absent by adding excess of phenylhydrazine dissolved in acetic acid to the cold hydrolysate and allowing the mixture to stand overnight and then filtering and heating the filtrate in a water-bath, when galactosazone only was formed.

The presence of galactose was further confirmed by preparing mucic acid both from the hydrolysate and from the unhydrolysed polysaccharide.

When the method of Nanji, Paton and Ling [1925] for testing and estimating uronic acids was applied no carbon dioxide was evolved, showing the absence of these bodies.

It is, therefore, concluded that mannose and galactose are the only sugars which enter into the constitution of this polysaccharide. The relative quantities of these two sugars were calculated as follows.

The specific rotatory power of the hydrolysate was $[\alpha]_D^{20} = 46.2^\circ$, the total concentration of the sugars being estimated by Bertrand's method and calculated as glucose.

If X is the quantity of mannose, then $1 - X$ = the quantity of galactose. We therefore have the equation

$$\begin{aligned} 14X + 81(1 - X) &= 46.2, \\ X &= 0.52. \end{aligned}$$

So that the quantities of mannose and galactose stand in the ratio 52 : 48 or the two sugars are present in almost equal quantities.

Bourquelot and Hérissé [1900] state that the reserve carbohydrate of fenugreek seeds is a mannogalactan. When, however, the polysaccharide was ignited it left an ash which was found to contain the following elements: iron, calcium, magnesium, phosphorus and silicon. The polysaccharide contains phosphorus in organic combination and, on hydrolysis with mineral acids, a gelatinous precipitate composed mostly of silica combined with a little carbohydrate separates out. The polysaccharide is, therefore, a salt of a silico-phosphoric ester of mannogalactan. The occurrence of such phosphoric and silicic esters of polysaccharides in nature has been reported by different authors; thus Pringsheim and Kusenack [1924] found that lichenin is a silicic ester, and in relation to the constitution of starch Ling and Nanji [1923] state that the amylopectin of the starch granules is a phosphoric ester, while Schryver *et al.* [1923] found that some starches contain a hemicellulose in which a certain amount of silica occurs in organic combination. Further Bourquelot and Hérissé reported that the mannogalactan of fenugreek was slightly different from that of lucerne.

From the above-mentioned description it seems that, apart from the constituent sugars, there is a close analogy in constitution between this polysaccharide and starch. The polysaccharide seems to be present in different forms and probably in different degrees of esterification with correspondingly different degrees of solubility like the amylose, amylopectin and amylohemiacellulose of the starch granules.

The digestibility of the polysaccharide.

Mannogalactans are hydrolysed by the enzyme seminase into mannose and galactose. This enzyme is not known to occur in the human body. Nevertheless the action of saliva, as well as that of an extract of the pancreas on the polysaccharide was studied. In both cases a 0.5 % solution of the mucilage was used to which varying concentrations of the digestive fluids were added. The activity of these fluids was always checked by using as a control 0.5 % starch paste. Judging by Fehling's reaction, the polysaccharide appeared not to have been influenced either by saliva or by the extract of the pancreas. In looking for the presence of sugars the polysaccharide was first precipitated with alcohol, the filtrate was evaporated on a water-bath to drive off the alcohol, and the residue was then tested for reduction. This course had to be followed because of the insoluble copper compound which the polysaccharide forms on the addition of Fehling's solution and which makes the detection of a small reduction very difficult or practically impossible.

Table I.

		Blood-sugar %			
		Subject F.		Subject M.	
Time		Bran	Whole meal	Bran	Whole meal
Fasting	...	0.091	0.105	0.090	0.088
$\frac{1}{2}$ hour after ingestion of polysaccharide	...	0.091	0.098	0.083	0.091
1	"	0.095	0.098	0.090	0.091
1 $\frac{1}{2}$	"	0.097	0.098	0.093	0.093
2	"	0.098	0.100	0.093	0.091
2 $\frac{1}{2}$	"	0.091	0.103	0.092	0.094
3	"	—	0.093	0.088	0.086
3 $\frac{1}{2}$	"	0.088	—	—	—
4	"	—	0.095	0.088	0.088
4 $\frac{1}{2}$	"	0.088	—	—	—
5	"	—	0.097	0.092	0.088

This result, which was expected, suggests that the polysaccharide may be a factor in the laxative effect which is supposed to follow the ingestion of these seeds. This would undoubtedly be correct if the polysaccharide is not affected by other agencies in the alimentary canal.

As an indirect way for finding out whether the polysaccharide is hydrolysed by other agencies in the alimentary canal its effect on the blood-sugar was studied. Samples of blood were taken in the morning from two fasting men immediately before, and half-hourly after, taking 50 g. of the bran which contains more than 75 % of the polysaccharide. In other experiments 50 g. of

the whole fenugreek seed meal, which contains 50 % of the carbohydrate, were given to the same men. The method of Hagedorn and Jensen for the estimation of blood-sugar was employed in these cases. The result obtained is given in Table I. In the case of subject F. there was a very slight but continuous rise of the blood-sugar when the bran was given. This is, however, of no significance, especially when the slight fluctuations in the other experiments are taken into consideration, and it follows that if the polysaccharide is attacked at all in the alimentary canal the products have no influence on the blood-sugar. If any change takes place it would be produced by bacteria in the lower intestine.

Another important point which can be inferred from the results of giving the whole fenugreek seed meal is that, if the dry seeds contain the specific enzyme or enzymes which are capable of producing the changes which occur in the polysaccharide during the germination of the seeds, these enzymes appear to remain inactive in the alimentary canal.

The polysaccharide during germination.

As the fenugreek seeds are administered, in some cases, in the germinated form, the fate of the polysaccharide during germination and the nature of its hydrolysis products under different conditions were fully investigated. Evidence of the suitability of different sugars for performing certain functions in nature was also gained.

When the powdered ungerminated seeds are extracted with 50 % alcohol, in order to dissolve any sugars present and at the same time prevent the polysaccharide from dissolving, and the liquid is filtered and evaporated, the extract does not reduce Fehling's solution and does not form an osazone even after hydrolysis. This proves that the seeds before germination do not contain sugars. The seeds are also quite free from starch.

When, however, the seeds are allowed to germinate, sugars make their appearance. It should be remarked that the photosynthetic process does not take part in the formation of these sugars because the observations are made before the cotyledons leave the testa or show any sign of turning green, and therefore these sugars are entirely due to the hydrolysis of the reserve polysaccharide itself.

Fenugreek seeds were steeped in water for 24 hours, the water was drained off and the seeds were placed in a shallow wooden box with perforated sides, covered with moist filter-paper, placed in a fairly warm place and moistened with water from time to time. After 48 hours' germination, the radicles were completely separated from the rest of the seeds. On grinding the radicles with fine washed sand to a paste and then extracting with water and filtering, the filtrate was found to reduce Fehling's solution strongly. When a portion of the filtrate was treated with phenylhydrazine and acetic acid and heated for 1 hour glucosazone only could be identified. The absence of mannose from the extract was repeatedly confirmed by obtaining no mannosephenylhydrazone on the addition of phenylhydrazine and acetic acid in the cold. The absence of galac-

tose was further confirmed by the failure to obtain mucic acid on oxidising a portion of the extract with nitric acid. This is an interesting fact because, whilst the reserve polysaccharide is composed of galactose and mannose, these two sugars do not make their appearance in the free state among other sugars during the germination of the seeds. Observations on the endosperm indicate clearly its gradual exhaustion with the advance of germination, which proves that the polysaccharide functions as a reserve and not merely as a structural material.

As the osazone obtained can be given by fructose as well, the Pinoff-Seliwanoff [Pinoff, 1905] test was applied, using at the same time control tubes containing a dilute solution of sucrose and a dilute and a more concentrated solution of glucose. The colour developed in 1 minute in the case of the extract and the control containing sucrose, whilst in the case of the glucose solutions it took more than half an hour to develop. The positive result with Pinoff's test, together with the fact that glucosazone was the only osazone obtained, suggest the possibilities of the presence of fructose alone, glucose together with fructose, glucose together with sucrose or glucose, fructose and sucrose.

After hydrolysis, the clarified extracts of the radicles show a marked increase in reducing power, and an osazone identical with glucosazone is again the only one obtained; further, the sign of rotation is changed from positive to negative. It is clear, therefore, that sucrose is present together with the reducing sugar or sugars. The simultaneous presence of both free fructose and glucose was made evident from the result of the following experiment.

An extract of the sugars from the dried powdered radicles was prepared by adopting the method of Bryan, Given and Straughn [see Browne, 1912] for the preparation of sugar solutions from plant substances.

The specific rotatory power of the extract was found to be $[\alpha]_D^{20} + 27.9^\circ$.

The reducing sugars, estimated by Bertrand's method and calculated as invert sugar, equal 0.485 %.

The reducing sugars, after hydrolysis calculated as invert sugar, equal 1.0675 %.

$$\therefore \text{sucrose} = (1.0675 - 0.485) \times 0.95 = 0.553 \%$$

It will be seen that the specific rotatory power of the extract cannot be accounted for except on the assumption that the reducing sugar consists of almost equivalent quantities of glucose and fructose, as is evident from the following calculation, considering half the reducing sugar as glucose and the other half as fructose, and multiplying the concentration of each sugar by its specific rotatory power, we obtain

$$0.242 \times 52.8 + (-92.5 \times 0.242) + 66.5 \times 0.55 = 27.16.$$

Thus the calculated rotatory power agrees with that observed and it is to be concluded, therefore, that the sugars present in the radicles are sucrose, glucose and fructose. The two latter are present in almost equivalent quantities.

Galactose and mannose were also proved to be absent from the cotyledons of the germinated seeds. It is evident that these two sugars are completely transformable into glucose, fructose and sucrose during germination. It is to be expected that the formation of both glucose and fructose precedes that of sucrose since linkages between the hexose residues of the type present in sucrose would be absent from a mannogalactan. It seems that the hydrolysis of the polysaccharide and the transformation of the constituent sugars into glucose and fructose proceed at a more rapid rate than that with which the latter two sugars are utilised for the liberation of energy, and consequently sucrose is formed as a temporary reserve.

The mechanism of the transformation of mannose and galactose into glucose and fructose is obscure. As regards the former, the transformation into its ketose analogue and its epimeride may to some extent be explained by the ease with which the reversal of the stereochemical configuration of carbon atom 2 takes place, but even then the complete absence of mannose during germination suggests a different mechanism. Still more difficult to understand is the transformation of galactose. The latter is also known to take place in the human body, thus it is stated that lactose when fed to diabetics may be recovered in the urine as two molecules of glucose for each molecule of lactose fed. This indicates that the tissues can under certain conditions transform galactose into glucose. This and similar transformations have been explained by Robinson [1927] to take place through the optical inversion on the hydrolysis of a hexose-4-phosphate.

Whatever the case may be, we are faced with the difficulty of explaining the reason why this transformation takes place in the germinating seeds only and not when the hydrolysis is effected by sulphuric acid. Work on this point is still proceeding in this Department.

There are, however, other possibilities as regards the non-appearance of galactose among the other sugars during germination, such as its accumulation in a polysaccharide-like form, which may become associated with the cell walls of the newly formed cells, or its preferential utilisation for the supply of energy. To clear up the first point, the ratio of the galactose to the rest of the reducing sugar produced by hydrolysing the ungerminated and the germinated seeds by dilute sulphuric acid was studied. There is some difficulty in experimenting with the germinated seeds as the hydrolysis of these gives rise to four sugars. The best procedure was to calculate the galactose from the yield of mucic acid obtained by oxidising the neutralised hydrolysate by nitric acid and to calculate the total sugar as galactose by Fehling's solution. Although the mucic acid method for the estimation of galactose usually gives low results, it serves for comparative purposes since the same experimental conditions were exactly followed in each case. In the case of the germinated seeds precaution was taken to avoid any loss of mucilage during steeping. The seeds were weighed out and were just covered with water in a porcelain basin and, when the water was absorbed, further quantities were added; after the absorption of

the last quantity the seeds became exposed to the air and were placed in a warm place to germinate. The germinated seeds were ground up with a small quantity of washed sand and then washed into a flask.

Taking equivalent quantities of the powdered ungerminated seeds and of the seeds used for germination, all other experimental conditions being the same, the ratios of galactose in the hydrolysate to the rest of the sugar calculated as galactose were as follows:

- (1) In the ungerminated seeds: galactose/the rest of the sugar = $1/2$.
- (2) In the germinated seeds: galactose/the rest of the sugar = $1/9$.

Supposing that the galactose accumulates in some form or other and none of it is transformed, its ratio to the rest of the sugar would increase during germination as some of the sugar in the case of the germinated seeds would be utilised for the supply of energy. That the galactose is not preferentially utilised for the latter purpose is evident from the fact that it is not present in the free state in any quantity during germination.

As regards the function of the polysaccharide it is seen that the combination of galactose with mannose results in the formation of a polysaccharide which not only functions as a reserve but which also possesses the property of imbibing large quantities of water and may possibly help early growth in situations where water is not plentiful. Further, in the dry state, this polysaccharide is hard and is therefore more efficient than starch for protecting the cotyledons, which are rich in nutritive material, from attack by insects and other external influences and, in fact, these seeds keep better than starchy seeds. Glucose and fructose appear to be the only easily assimilable sugars which are utilised for the supply of energy, whilst when they are in excess they are stored in the form of a temporary local reserve in the form of sucrose.

SUMMARY.

(1) The reserve polysaccharide of fenugreek seeds is a salt of a silico-phosphoric ester of mannogalactan. The two sugars entering into its constitution are present in almost equivalent quantities.

(2) The polysaccharide is not hydrolysed by the saliva or the pancreatic extract *in vitro*, and if it is assimilated from the alimentary canal the products show no influence on the blood-sugar.

Whole fenugreek meal, like the isolated polysaccharide, does not cause a change in the blood-sugar.

(3) During the germination of the seeds the mannose and the galactose constituents of the reserve polysaccharide are transformed into glucose, fructose and sucrose. Nothing is known of the mechanism of this transformation.

In conclusion, I desire to express my thanks to Dr A. Hassan for suggesting the work and for his encouragement and advice.

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