XXVII. THE MECHANISM OF THE INTER- CONVERSION OF THE HEXOSE SUGARS IN LIVING ORGANISMS.

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THE transformation of one hexose sugar into another is a process which takes place in both kingdoms of living matter. Thus in animals, fructose, mannose and galactose when slowly introduced into the circulation form glycogen which is supposed to be a result of an intermediate formation of glucose. When lactose is administered to diabetics two molecules of glucose for each molecule of lactose may be recovered in the urine. This indicates that the tissues can under certain conditions transform galactose into glucose. The reverse process, that is the transformation of glucose into galactose, has been observed by Rohmann [1919], who shows that the galactose constituent of milk sugar is formed from *d*-glucose.

In plants, Daoud [1932], working on the germination of fenugreek seeds, found that while the reserve polysaccharide of these seeds is composed of mannose and galactose, the radicles of the germinated seeds contain glucose, fructose and sucrose, this last sugar being entirely absent from the ungerminated seeds. The reverse process undoubtedly takes place during the development of the seed, *i.e.* the galactose and the mannose constituents of the reserve polysaccharide must arise from the photosynthetic sugars.

The mechanism of the transformation of one hexose into another is the subject of the present investigation. The transformation of a sugar such as mannose into its epimeride (glucose) may to some extent be explained by the ease with which the reversal of the stereochemical configuration of carbon atom 2 takes place. This occurs *in vitro* under the action of alkalis on either of these two sugars; but as a result of this reaction a mixture of these three and fructose together with other sugars is formed. The fact that the mannose constituent of the reserve polysaccharide of fenugreek seeds is not found in the free state among the other hexoses during germination suggests that the mechanism *in vivo* is different from that *in vitro*. Still more difficult to understand is the transformation of a sugar like galactose into glucose or *vice versa*.

Robinson [1927, 1], bearing in mind the common occurrence of hexose-phosphates in nature, suggests that the transformation of glucose into galactose takes place through the reversal of the stereochemical configuration of carbon atom 4 on the hydrolysis of glucose-4-phosphate; the reverse change will have the same mechanism.

The view of Robinson was opposed by Levene [1927] who observed that glucose-3-phosphoric acid ferments at the same rate as glucose. Should glucose-3-phosphoric acid on hydrolysis pass into allose, then the rate of fermentation would be lower than that of glucose. Furthermore, Raymond and Levene found that on hydrolysis of glucose-3-phosphoric acid, only one sugar, namely glucose, is obtained.

Robinson [1927, 2] brings into consideration firstly, that even if his hypothesis of inversion in the hydrolysis of sugar phosphate in nature is correct it may well be difficult to find the conditions for the imitation of the process in the laboratory, and secondly, that the glucose-galactose change involves position 4 where the stereochemical conditions are different from those in the glucose-3-phosphoric acid used by Levene.

Levene *et al.* [1929] found, in favour of Robinson's suggestion, that the hydrolysis of the phosphoric group in fructose-3-phosphoric acid during osazone formation is accompanied by a Walden inversion.

It may be remarked that the reserve polysaccharide of fenugreek seeds, a detailed study of the behaviour of which is provided in this communication, is a mannogalactan which is also esterified by phosphoric and silicic acids. On germination it gives rise to glucose and fructose, while, apart from the seeds, hydrolysis by different means results only in the production of galactose and mannose. A hydrolysis of the original ester takes place in all cases.

Mathers and Robertson [1933], working on the hydrolysis of *p*-toluenesulphonyl esters of glucose, were able to convert a derivative of glucose into a derivative of altrose. Cognate researches by Robertson and Oldham [1934] provided evidence that the hydrolytic reactions fall into two main groups, normal and abnormal, according as the original substance contains one or two *p*-toluenesulphonyl residues in neighbouring positions. They have also shown that in cases in which Walden inversion has been proved to occur, such inversion is accompanied by anhydro-formation. According to them the anhydro-formation may be a necessary precursor of this type of inversion, which follows as a consequence of the opening of the anhydro-ring.

Lohmann [1933] has shown that in yeast and animal cellular extracts there is an agent which converts both ketohexose- (Neuberg ester) and aldohexose-monophosphoric ester into Embden ester which is an equilibrium mixture composed of aldo- and keto-hexosemonophosphoric esters in which the former are present to a predominant extent. It may be noted that this investigation applies only to the transformation of an aldose into its ketose analogue or *vice versa*.

Robison [1932] and Macleod and Robison [1933], working on the products of yeast fermentation and on the hydrolysis of hexosediphosphate produced during the fermentation, suggest schemes which may possibly apply to the transformation of one hexose into another. According to these schemes an enolic compound common to glucose, fructose and mannose is formed, which reacts with phosphoric acid and the resulting enolic phosphate passes into a mixture of esters of the three above sugars. But, again, these schemes suggest only the mechanism of the interconversion of a sugar with its epimeride and its ketose analogue and, if applied to the problem under investigation as a whole, they give no clue to the transformation of a sugar like galactose into glucose or fructose as happens during the germination of seeds.

EXPERIMENTAL.

It has already been mentioned that the sugars entering into the constitution of the reserve polysaccharide of fenugreek seeds are mannose and galactose, which are transformed during germination into glucose, fructose and sucrose. It is natural to attribute such transformations to the influence of enzymes elaborated by the seeds. As the transformation may be brought about by the co-operation of more than one enzyme, it was decided to study the action of the enzymic mixture of the germinated seeds on the polysaccharide *in vitro*.

The enzyme preparations.

Fenugreek seeds, in different stages of germination, were used for the preparation of the enzyme mixture. The seeds were first minced in a meat mincer and ground up in a mechanical mortar into a fine paste, which was then extracted either with chloroform water or with glycerol. The proportion of solvent in each case was 1 ml. per g. of paste. The paste was allowed to stand with either solvent for different intervals of time varying from 6 hours to 10 days and was shaken from time to time. For some experiments the paste was treated with distilled water and was mechanically shaken for $2\frac{1}{2}$ hours after which it was prepared for immediate use. For use the mixture of paste and any of the solvents used was placed in a calico bag and pressed out by hand, followed by a press. The liquid obtained by centrifuging the fluid was used in the experiments. In some experiments the enzyme mixture used was prepared by treating the paste with a small amount of water and a little toluene and then allowing to stand for a few days in an incubator in order to allow the cells to autolyse and to liberate their enzymes. Water was then added to the mass which was subsequently treated as above.

Action of the mixture of enzymes on the polysaccharide in vitro.

It should be remembered that the mixture of enzymes prepared by any of the above methods contains, in case of short extractions, glucose, fructose and sucrose. After prolonged extraction the amount of sucrose diminishes, finally leaving only the two former sugars. This is due to the sucrose undergoing hydrolysis by the invertase which is present in the extract. The preparations also contain the polysaccharide which had not been utilised during germination, and also, in the case of long extractions, its hydrolytic products.

The quantity of the enzymic solution employed was usually 2–3 ml. for every 40 ml. of the polysaccharide solution; thus the amount of the sugars originally present in the reaction mixture due to the enzymic preparations was too small to influence the identification of the sugars resulting from the hydrolysis of the polysaccharide. Nevertheless, controls with the same quantity of enzyme solution added to a quantity of water equivalent to the volume of the polysaccharide solution used were performed at the same time and treated in a similar manner.

The purified polysaccharide of fenugreek seeds, which is a silicophosphoric ester of mannogalactan, was prepared by the method of Daoud [1932]. Different antiseptics (toluene, camphor, thymol, chloroform and sodium fluoride) were used in the different experiments.

When the mixture of enzymes, prepared by any of the above-mentioned methods, was added to the polysaccharide solution together with one of the antiseptics and incubated at 37° , it was noticed that the opalescence as well as the viscosity of the solution gradually diminished with simultaneous deposition of an insoluble residue, while the reducing power gradually increased. The insoluble residue was found to be composed mostly of inorganic constituents including silica, phosphates, iron, calcium and magnesium. Portions of the reaction mixture, removed at different intervals of time, were treated with an equal volume of 95 % alcohol; the precipitate of polysaccharide obtained showed a gradual diminution in quantity and gradual changes in property as the reaction proceeded. The precipitate obtained as soon as the enzyme was added was a white filamentous mass, while that obtained at advanced stages of the reaction was white and pulverulent and dissolved in water with greater ease.

The changes taking place during the action of the mixture of enzymes appear to be as follows: firstly, there is a hydrolytic removal of the silicic and phosphoric acids, which is most probably brought about by esterases with the production of a more soluble substance, and secondly, a simultaneous or subsequent hydrolysis of the de-esterified substance into sugars. There is also the possibility that the more soluble substance includes degradation products of the original polysaccharide. The de-esterification proceeds at a greater rate than the hydrolysis into sugars since the precipitate of silica and phosphates produced during the enzymic action ceases to increase after a certain period while the reducing power continues to increase further.

To investigate the nature of the sugars produced by the action of the enzymes on the polysaccharide, the following procedure is adopted.

The reducing power is determined from time to time by removing portions of 25 ml. of the reaction mixture, making up to 50 ml. with 95 % alcohol, filtering, washing with 50 % alcohol, evaporating the alcohol, making up the residue to the original volume with water and determining the amount of reducing sugars by Bertrand's method.

When the reducing power shows little or no further increase, the whole of the reaction mixture is removed from the incubator, filtered from the insoluble residue, shaken with a small amount of calcium carbonate and refiltered. The filtrate is treated with alcohol to precipitate any traces of polysaccharide which sometimes remain unattacked, refiltered if necessary and finally concentrated in a vacuum to a smaller volume than the original. This somewhat concentrated solution is treated with normal lead acetate until maximum precipitation is reached, any excess of lead being removed by sodium carbonate; the filtrate after removal of the lead is neutralised with acetic acid and decolorised.

When portions of the solutions thus obtained are heated with phenylhydrazine hydrochloride and sodium acetate, two osazones are obtained, one of which is identical with glucosazone and the other with galactosazone. The presence of galactose is further confirmed by the production of mucic acid on oxidising a portion of the solution with nitric acid. On treating other portions of the solutions with phenylhydrazine and acetic acid in the cold a white crystalline precipitate is obtained which is identified by its crystalline form and its melting-point as mannosephenylhydrazone. The glucosazone obtained on heating, with the exception of a very small amount which is due to the glucose and fructose content of the enzyme solution, is entirely due to mannose and not to glucose or fructose which might have been formed during the breakdown of the polysaccharide. This is proved by adding an excess of phenylhydrazine dissolved in acetic acid to a portion of the cold solution and keeping the mixture in the ice-chest for 24 hours, then filtering off the mannosephenylhydrazone, heating the filtrate in a water-bath, and examining the osazone thus formed after the removal of the mannose. It is found that the main osazone present is galactosazone together with a very small amount of glucosazone which is as above-mentioned due to the sugars of the enzyme solution. Further, quantitative experiments prove beyond any doubt that the sugars produced by the enzymic action on the polysaccharide are entirely mannose and galactose. These quantitative experiments are performed as follows.

A measured volume of the polysaccharide solution is treated with a known volume of one of the enzymic preparations. At the same time a control is made by the addition of the same volume of the same enzymic preparation to a volume of water which is equal to the volume of the polysaccharide solution employed. Both solutions are incubated till no precipitate of polysaccharide is produced

on the addition of alcohol to a portion of the polysaccharide solution and they are then filtered and equal volumes of the filtrates are clarified and decolorised, taking equal measured volumes of the filtrates after each process. The mannose in the final solutions is estimated as mannosephenylhydrazone. After allowing for the quantity of mannose in the control, it is found that in all cases the yield of mannose from the polysaccharide alone by the action of the enzyme almost agrees with the theoretical amount of mannose which is calculated to be present in the quantity of polysaccharide originally taken. The amount of mannose found is never less than 98.8 % of the theoretical amount.

The above solutions are also used for calculating the specific rotatory power of the mixture of sugars resulting from the enzymic hydrolysis of the polysaccharide. The percentage of reducing sugars calculated as glucose and also the rotation of the final solution in the case of the control are subtracted from those of the final solution derived from the polysaccharide. The balance of the rotation is considered to be due to the amount of sugars resulting from the enzymic action on the polysaccharide only. The specific rotatory power calculated on this assumption for the several experiments performed varies from $[\alpha]_D^{20} + 45.9^\circ$ to $[\alpha]_D^{20} + 48.1^\circ$. The theoretical specific rotatory power for a mixture of equal quantities of galactose and mannose is $[\alpha]_D^{20} + 47.5^\circ$.

Since the yield of mannose from the polysaccharide, which contains equal quantities of mannose and galactose, is quantitative, and these two sugars are produced by the enzymic action in equal quantities, it follows that the only products of the enzymic action on the polysaccharide are galactose and mannose.

It was noticed that the extracts of the germinated seeds were usually slightly acid to litmus. This observation suggested that the hydrogen ion concentration might have an effect in causing the interconversion of the hexoses to take place in the germinating seeds. The polysaccharide solution was buffered at p_H values varying between 5.4 and 7.8. It was found that the production of galactose and mannose from the polysaccharide by the action of the enzymic mixture is independent of the hydrogen ion concentration of the medium within these limits.

The above observations lead to the conclusion that the hydrolytic products of the reserve polysaccharide of fenugreek seeds are always the same whether the hydrolysis is effected by acids or by the enzyme mixture from the germinated seeds *in vitro*.

Direct investigations on the germinating seeds.

It was noticed that while mature ungerminated fenugreek seeds are usually starch-free, the germinated ones contain starch and that this increases in amount with the progress of germination. Some specimens of fenugreek seeds were found to contain traces of starch before germination; this, however, is rare and is most probably due to an immaturity of the seeds or an imperfect drying and preservation of the harvest. It was proved by various methods that the substance formed during germination is starch. In some cases the starch appeared to have an admixture of some other substance probably of the nature of an erythro-dextrin, since on the addition of iodine to the isolated substance a violet coloration instead of blue was sometimes given.

The starch formed was not the result of photosynthesis as in all cases the investigations were made before the cotyledons left the testa or showed any sign of chlorophyll formation. Furthermore the steeping and the germination were carried out in darkness.

The importance of determining the relation of the sugars, especially that of glucose, appearing during germination to the starch is apparent. An attempt

was therefore made to find out which of these compounds first appears in the germinating seeds. This presents certain difficulties since in most seeds starch and sugars seem to occur practically at the same time at the beginning of the germination. On carefully following the formation of starch in starch-free fenugreek seeds, it was observed that this substance appears during steeping; usually after 10 to 18 hours of steeping. The following procedure was therefore adopted to find out which of the above substances appears first.

A weighed quantity of the seeds steeped till the starch became detectable was thrown into a small volume of vigorously boiling water to stop all enzymic action. Boiling was continued for one hour. The seeds were then drained, weighed, ground into a paste and remixed with the drained water. Alcohol was added to this mixture to a concentration of about 50 %, taking into consideration the quantity of water absorbed by the seeds during the steeping. Calcium carbonate was added to neutralise the solution which was then boiled on a water-bath under reflux for 2 hours in order to extract any sugars formed. The filtered extract was distilled *in vacuo* to a small bulk. The concentrated residue was clarified and examined for reducing sugars by the osazone test. In the great majority of the experiments no osazone could be detected. In very few exceptional cases small traces of glucosazone were found. The amount was exceedingly small if it be remembered that we dealt in each case with a concentrated extract of 100 ml. obtained from 1 kg. of the dry seeds. This result indicates that the formation of starch precedes that of glucose. It has been observed that while the reserve mannogalactan of fenugreek seeds is contained in the endosperm, the formation of starch takes place entirely in the cotyledons and radicles. The amount of starch in these organs increases with the exhaustion of the endosperm, thus indicating the formation of starch at the expense of the reserve mannogalactan.

Further support of the view that the starch formation precedes that of the glucose was gained by observations made on single germinating seeds of carob (*Ceratonia Siliqua*). The reserve polysaccharide of these seeds is also a mannogalactan which, as in the case of fenugreek seeds, gives glucose, fructose and sucrose during germination. These seeds are difficult to germinate under laboratory conditions as they possess a very hard testa and a thick hard endosperm, while the cotyledons are very thin and practically free from carbohydrates. The straight radicles of these seeds allowed easy measurement which helped in determining the beginning of germination. Germination was only possible after steeping for a long time and then carefully removing the testa and the mucilage layer round the radicle. The germination was still slow even after exposing the radicles. In order to have the cotyledons accessible for the purpose of detecting the first appearance of starch, the testa at the top of the seeds was removed. The seeds were always kept moist during germination. Every 12 hours a minute fragment of one of the cotyledons was removed at the top of the seed by means of a thin needle and tested with iodine under the microscope. It was found that starch makes its appearance in the cotyledons before the radicles show any increase in length. Elongation of the radicles was almost simultaneous with the appearance of reducing substances in them; both take place between 24 and 36 hours after the first appearance of starch. It was further noticed that some seeds failed to form starch and these never germinated.

It is, therefore, justifiable to assume that the mannogalactan of carob and fenugreek seeds gives rise to starch and that the glucose which appears during germination arises as a result of hydrolysis of the starch by an amylase which has been experimentally found to be elaborated during germination.

The experiments just described gave a clue to the probable course followed in the formation of the reducing sugars found in the radicles of the germinating seeds, sugars which are entirely different from those constituting the reserve. If the starch supplies the glucose, another compound might be expected to be responsible for the supply of fructose. Other polysaccharides were, therefore, looked for. The concentrated clarified extracts obtained from the steeped fenugreek seeds by the method described before were treated with alcohol. A precipitate began to appear when the concentration of alcohol was about 64 % and gradually increased with the addition of more alcohol till the concentration reached 75 %. The small precipitate formed was collected by centrifuging, redissolved and reprecipitated three times, treated with absolute alcohol followed by ether and dried *in vacuo*. The white powder so obtained was readily soluble in water, did not reduce Fehling's solution and was, in most cases, laevorotatory. In a few cases, however, the substance had a feeble dextrorotation. The variation in rotation suggested a non-homogeneous constitution for this substance. The product was, therefore, subjected to fractional precipitation. It was found that a portion came down when the concentration of alcohol was about 64 %. This was collected by centrifuging and proved to be a pentosan. When the concentration of alcohol in the remaining fluid was raised to 70–75 % a second minute fraction was obtained. This fraction was white when dry, tasteless, did not reduce Fehling's solution and was laevorotatory. When hydrolysed with 1 % sulphuric acid under reflux on a boiling water-bath for half an hour, the hydrolysate after neutralisation with barium carbonate and filtration reduced Fehling's solution, gave an osazone identical with glucosazone, yielded the Pinoff-Seliwanoff's reaction and had a laevorotation. The specific rotation of the hydrolysates obtained from different experiments varied from $[\alpha]_D^{20} - 89.7^\circ$ to -91.2° . The specific rotation of fructose is $[\alpha]_D^{20} - 92.5^\circ$.

This clearly indicates that the sugar obtained on the hydrolysis of the above fraction is fructose, and that the original substance is composed of polymerised fructose residues and is similar to, if not identical with, inulin. It will be referred to as fructosan.

After removal of the fructosan, the fluid still contained a substance of a carbohydrate nature which came down only when the concentration of alcohol was very high. This rendered its separation by this method inconvenient. This substance was found to form a precipitate with Fehling's solution which was used for its separation. The alcohol contained in the fluid after the removal of the two above-mentioned fractions was distilled and Fehling's solution added. The plastic precipitate formed was rapidly collected, dissolved in a little dilute hydrochloric acid and precipitated with acetone. The precipitate was collected, redissolved, reprecipitated with acetone three times and finally dried. The dried substance is pale-yellow in colour, readily soluble in water with frothing, it has a bitter taste and causes a slight reduction of Fehling's solution when heated or in the cold if allowed to stand for a sufficient time; it gives no osazone and is laevorotatory. When its solution is hydrolysed, it shows a dextrorotation, its reducing power greatly increases, while a considerable amount of precipitate is formed. This shows that the substance behaves in every respect like a saponin; further it gives similar colour reactions and causes haemolysis.

As this saponin was found to be present in the dry ungerminated fenugreek seeds and as it was proved to be utilised during germination, it became necessary to determine the nature of its sugar content. On hydrolysis galactose and mannose were the only two hexoses identified.

All the above experiments show that the reserve carbohydrates of fenugreek

seeds are entirely constituted of mannose and galactose and that the whole glucose and fructose which appear during germination are to be regarded as transformation products.

The identification of the minute amount of fructosan in the steeped seeds necessitated the examination of the ungerminated seeds for the presence of that substance. Powdered ungerminated fenugreek seeds were extracted with 50 % alcohol following the steps already mentioned in the extraction of the steeped seeds. When the clarified solution was treated with alcohol, the precipitate obtained between 64 and 75 % concentration was in all cases dextro-rotatory.

When alcohol was added to the clarified solution to a concentration of 75 % the precipitate obtained, contrary to the case of the steeped seeds, could not be fractionated into a pentosan and a laevorotatory body. Pentoses were the only products obtained on hydrolysis. This shows that the fructosan is not originally present in the ungerminated seeds, but that, similarly to starch, it is formed only during steeping.

Examination of the seeds in different conditions for the presence of an enzyme which attacks inulin has shown that while extracts of the ungerminated seeds have no action on that substance, those of the steeped and germinated seeds feebly attack it. The secretion of an enzyme which attacks inulin simultaneously with the formation of the fructosan in the steeped or germinated seeds suggests that the fructosan formed is built up on lines similar to inulin.

Since no glucose or fructose is formed in a free state during the period of steeping, it becomes clear that both the starch and the fructosan formation from the galactose and the mannose constituting the reserve polysaccharide of the ungerminated seeds must precede the appearance of glucose and fructose of the germinated seeds. This explains the failure of the enzymic mixture of the germinated seeds to produce the interconversion of the hexoses of the reserve polysaccharide *in vitro*.

Having demonstrated that starch and fructosan appear in the steeped seeds before the appearance of reducing sugars, the behaviour of these two polysaccharides was next studied during different stages of germination of the fenugreek and other seeds. The results obtained can be summarised as follows. On germination the starch gradually increases in amount; the fructosan can be detected in fenugreek seeds during all stages of germination but always in small quantities, it seems to diminish further or to disappear on prolonged germination. In carob seeds the fructosan could not be detected at all. The following table illustrates the changes in the quantities of the starch and the fructosan during different stages of germination of fenugreek seeds. The figures are expressed in g. per 100 g. of the dry seeds.

Exp.	Time	Starch	Fructosan
I	Before steeping	0.00	0.00
	1 day germination	1.48	0.067
	2 days	2.44	0.071
	4 "	4.15	0.054
	6 "	5.97	0.035
	8 "	7.71	0.021
II	8 "	8.02	Practically nil
III	8 "	8.31	0.014

It has already been mentioned that, besides the glucose and the fructose, sucrose is also formed during the germination of fenugreek seeds. Examination of seeds similar to those of fenugreek as regards their reserve polysaccharides

and of other seeds containing different forms of reserve polysaccharides, such as galacto-araban and starch, proved that sucrose is always produced during germination. Experiments to show the relation between sucrose and the reducing sugars glucose and fructose were performed by quantitatively determining the amounts of these sugars at the early stages of germination in the different seeds examined. This was done by extracting the three sugars with boiling 80 % alcohol, taking all the precautions already mentioned in investigating the relation between starch and glucose. The results of these experiments as well as the significance of sucrose and the part it possibly plays in the mechanism of the interconversion of the hexoses will be referred to in the discussion.

DISCUSSION.

It is clear from the foregoing experiments that formation of starch and a fructosan from the galactose and the mannose of the ungerminated seeds precedes the appearance of glucose and fructose in the germinated seeds. It would appear therefore that the transformation of one hexose into another occurs through an intermediate formation of a polysaccharide which gives rise to the new sugar on hydrolysis. When the radicles of dry non-starchy seeds are removed and the residual parts of the seeds are steeped and incubated at the same time with similarly treated seeds but with radicles left intact, neither starch nor sugars appear in the former while considerable amounts of both appear in the latter.

The work of Rohmann [1919] affords further evidence that in the process of interconversion there is an intermediate polysaccharide formed which gives rise to the new sugar. He found that the mammary gland contains a substance not identical with glycogen from which lactose may be obtained by ferment action. He points out that this substance is formed in the milk gland out of *d*-glucose and that on hydrolysis of this complex substance lactose is produced. It follows that the galactose constituent of the lactose molecule must arise from *d*-glucose during the polysaccharide formation.

Taking into consideration the work of the different investigators, including the present work, it can be concluded that this mechanism, if not universal amongst living organisms, plays at least a conspicuous part in the interconversion of hexoses.

Thus, according to this point of view, the actual transformation of one sugar into another takes place during the polymerisation of the hexose residues of the sugar undergoing transformation to form the polysaccharide which ultimately gives rise to the new sugar. That the process does not involve a direct transformation of one polysaccharide into another is deduced, firstly, from the presence of a powerful enzyme in the seeds which hydrolyses the original polysaccharide into its constituents and, secondly, from the fact that the starch formation in endospermic seeds takes place in the cotyledons and radicles but not in the endosperm where the original polysaccharide is present. Since the diffusion of colloidal matter through cell membranes is not likely to take place it is more probable that it is the constituent sugars of the original polysaccharide which diffuse into the embryo and polymerise to form the new polysaccharide. In order to see how this agrees with the well-known structural and other characteristics of sugars and polysaccharides, the following may be brought into consideration.

The difference between galactose and glucose lies in the configuration of carbon atom 4 and it has been shown by Haworth and Percival [1931] that in

starch, the glucose residues are linked together by an α -linkage joining the fourth carbon atom of one residue with the terminal carbon atom 1 of another residue. It is seen, therefore, that it is the same carbon atom 4, the configuration of which determines the difference between galactose and glucose, which is concerned in the linkage of the hexose residues. This indicates the possibility of the stereoisomeric change taking place on the polymerisation of the residues to form a polysaccharide.

In the transformation of a sugar like mannose or glucose into fructose, one hydrogen atom on carbon atom 2 must change position with the oxide ring on carbon atom 1. In inulin, the mode of linkage of the fructose residues has been determined by Drew and Haworth [1928]. According to these authors the junction of pairs is between the reducing group 2 of one residue and the terminal group 1 of another residue, while the oxide ring connects positions 2 and 5 in each residue; thus according to this inulin is composed of γ -fructose residues. In view of the appearance of the enzyme inulase in the germinating seeds of fenugreek, the fructosan formed during germination is supposed to be built up on similar lines to inulin. Whatever the constitution of the fructosan may be, it is evident that in a non-reducing polysaccharide composed of fructose residues the reducing group 2 must be concerned in the linkage of residues. It is seen, again, that the carbon atom concerned in the linkage of the residues is the atom at which the rearrangement takes place, and it is quite possible that such a rearrangement between a hydrogen on carbon atom 2 and the oxide ring on carbon atom 1 in glucose or mannose is effected by the linkage of carbon atom 2 to another hexose residue on polymerisation of the residues for the formation of the fructosan.

It may be remarked that the fructosan formed during the steeping and the germination of fenugreek seeds is either a highly labile substance or a substance of no great importance for the mechanism of fructose formation since, contrary to starch, it is only found in small quantities at any stage of germination. Further the experiments show that on prolonged germination the fructosan diminishes considerably or even vanishes, and that in seeds of similar reserve, as well as some seeds of different forms of reserve, it could not be detected. On the other hand sucrose is formed in considerable amounts during the germination of seeds containing different forms of reserve polysaccharides. In early stages of germination of seeds in which the fructosan was not identified it has been found that when the reducing sugars just become detectable the amount of sucrose is considerably greater than that of reducing sugars. Had the sucrose been formed at the expense of the reducing sugars glucose and fructose such a result would not be expected. Further, with the production of sucrose there is also a production of a strong invertase. These observations indicate the possibility of the sucrose playing a part in the mechanism of fructose formation. The fructose residue of sucrose can arise from mannose or from glucose on condensation with another glucose residue in a manner similar to that described for the fructosan formation. Again Haworth [1920] has shown that the linkage between fructose and glucose in sucrose is through their reducing groups, the fructose residue being present in the γ -form, i.e. in the formation of sucrose the carbon atom 2 of one hexose residue is concerned in the condensation.

The following two possibilities of the transformation of a hexose into a polysaccharide which subsequently gives rise to another hexose suggest themselves:

- (1) A stereoisomeric change which takes place at the carbon atom concerned in the linkage of the hexose residues as, for example, the transformation

of galactose into glucose through starch or *vice versa*, *i.e.* glucose into galactose through a polymeride like the reserve polysaccharide of some seeds.

(2) Intramolecular rearrangement between a hydrogen atom on carbon atom 2 and the oxide ring on carbon atom 1 in the same residue. This takes place in the transformation of an aldose into its ketose analogue as, for example, in the transformation of glucose or mannose into fructose through a polymeride of the latter or its condensation product with another hexose forming a higher sugar. The reverse rearrangement may take place in the transformation of fructose into mannose or glucose through polysaccharides like starch, glycogen or compounds containing mannose.

The possibility of the transformation of aldoses into polymerides of ketoses has been indicated by Colin [1918], who showed that inulin was formed in places of storage by condensation of the sugars synthesised by the leaf.

Evidence of the transformation of fructose into glucose through the intermediate formation of glycogen can be gained from the work of several investigators, *e.g.* Wittgenstein [1923]; Cori [1926]; Cori and Cori [1928] and Corley [1929]. The work of these authors leads to the conclusion that fructose requires transformation into glycogen before it can be utilised. In the formation of glycogen, of starch and generally of non-reducing polysaccharides composed of aldose units, the junction of pairs of units must be through the terminal reducing group of one residue and some other group in the other residue. It is, again, clear that the carbon atom 1 which is concerned in the junction of units is the atom at which the rearrangement takes place between the oxide ring on carbon atom 2 of fructose and a hydrogen atom on carbon atom 1. The transference of the hydrogen atom to carbon atom 2 may lead to the production of either glucose or mannose residues in the resultant polysaccharide. This assumption postulates that in the transformation of glucose or mannose into fructose or *vice versa* the oxide ring in either case moves to the carbon atom concerned in the linkage of pairs of units.

The consideration of the structure of the fructose residue in the natural plant products gives further support to these views. It can be concluded from recent investigations that fructose is the only sugar known to exist in the γ -form in its polymeride inulin and its condensation products like sucrose. According to the mechanism under discussion, when a sugar like glucose or mannose is transformed into a polymeride of fructose or a condensation product of it with another sugar, the ring which is an amylenic oxide ring in the original sugar, *i.e.* joins positions 1 and 5, will now join positions 2 and 5, *i.e.* becomes a butylenic oxide ring. This may give an explanation of the natural existence of fructose in the γ -form in its polymerides or condensation products only.

It remains to explain, on similar grounds, the transformation of mannose into glucose. During the present work evidence has been obtained that such a transformation takes place. The only carbohydrate reserve of carob seeds (*Ceratonia Siliqua*) is a polysaccharide composed of mannose and galactose similar to that of fenugreek seeds, but it has been found that the two sugars exist in the proportion of four of the former to one of the latter, while in fenugreek seeds the two exist in equal quantities. When the seeds were germinated, glucose, fructose, sucrose and starch were formed. Examination of the radicles proved that the glucose and the fructose existed in almost equivalent quantities; the sucrose also contains these two constituents in equivalent quantities and in addition to that, starch is formed which is composed entirely of glucose residues. If we consider that the glucose arises from galactose only, the glucose content of the germinated seeds would not exceed one-fifth of the

total hexose content of the different above-mentioned compounds. The fact that the glucose element is in excess over the fructose one can only be explained if we assume that mannose also gives rise to glucose.

Applying the mechanism under discussion to the transformation of mannose into glucose, we must consider that starch in the higher plants and glycogen in the lower plants and animals are intermediate compounds. The difference between mannose and glucose lies in the configuration of the second group of the hexose molecule; but in the formation of a compound like starch from hexose residues the fourth carbon atom is the one concerned in the linkage of pairs. Unless we know more about the nature of the polysaccharides and the mode of polymerisation no explanation can be given with certainty, but two possibilities suggest themselves.

(1) A formation of an intermediate polysaccharide composed of glucose residues in which the junction of pairs is at the carbon atom 2 of one residue and the terminal carbon atom 1 of another residue. Such a polysaccharide has not been identified in the steeped or germinated seeds.

(2) A more probable explanation which fits to some extent with experimental facts is that the mannose is transformed into a fructosan or gives rise to a fructose residue on condensation with another hexose forming a non-reducing reserve sugar such as sucrose. These fructose-containing compounds give rise to fructose which undergoes polymerisation forming a glucose precursor such as starch or glycogen. This assumption is supported by the observation already mentioned that the fructosan found in steeped and germinated fenugreek seeds always occurs in small quantities and further diminishes or even vanishes with prolonged germination. Secondly, in all non-starchy germinated seeds the glucose element in its different forms, especially starch, is found in considerable excess over the fructose element. These observations tend to show the transformation of fructose and its compounds into starch. In this respect, it is of interest to mention that Colin and Belval [1922; 1923] working on the genesis of carbohydrates in wheat, found that a fructosan is present in the stem during the flowering season and in the seeds a little after that season. This fructosan gradually disappears with the maturity of the seeds, thus indicating the formation of starch at the expense of this compound.

SUMMARY.

1. An important mechanism of the isomeric change of one hexose sugar into another in the living organism is the formation of an intermediate polysaccharide or a condensation product such as a non-reducing sugar which gives rise to the new sugar on hydrolysis.

2. It is suggested that the actual transformation of the sugar takes place on polymerisation or condensation in one of two ways. Either a reversal of the stereochemical configuration of the carbon atom concerned in the linkage of pairs of the hexose residues, or an intramolecular rearrangement between the oxide ring and a hydrogen atom on the first and second carbon atoms of the hexose molecule. The oxide ring moves to the carbon atom concerned in the linkage of pairs of residues. The former case occurs in the transformation of a sugar into its stereoisomeride, *e.g.* galactose into glucose or *vice versa*, and the latter case during the transformation of a sugar into its ketose analogue, *e.g.* glucose or mannose into fructose or *vice versa*.

3. An explanation of the natural existence of fructose in the γ -form in its polymerides such as inulin or its condensation products such as sucrose can be

given on the basis of these views. It is assumed that such plant products originate from aldoses, such as glucose, of the photosynthetic process on polymerisation or condensation.

4. The influence of the living organism is a determining factor in the process of interconversion since the elaboration of the intermediate polysaccharides does not take place in the absence of the organism. When the enzymic extracts of germinated seeds in which interconversion of the hexoses takes place are allowed to act on the reserves of these seeds *in vitro*, the end products obtained are identical with those obtained by acid hydrolysis.

If the vitality of the seeds is destroyed by removing the radicles, then on steeping and incubating the seeds, no intermediate products or sugars are formed.

5. Together with the production of the intermediate compounds in seeds there is also a production of the specific enzymes which attack them.

6. Starch, besides playing a part in the mechanism of the interconversion of the hexoses, is usually the principal reserve carbohydrate of seeds. In starch-free seeds the different forms of carbohydrate reserves are transformed during germination mainly into starch. The other intermediate compounds playing a part in the interconversion of hexoses are formed in much smaller amounts than starch and are possibly transformed into it. The process is reversible and starch in its turn can give rise to other forms of reserves when necessary.

In starch-free endospermic seeds the starch formation takes place in the cotyledons and radicles at the onset of metabolic processes but not in the endosperm where the original reserve carbohydrates mainly exist.

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