34. SOLUBLE RESERVE-CARBOHYDRATES IN THE LILIIFLOREAE

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CARBOHYDRATES are often stored in plants to be used in subsequent metabolic processes. Cell membranes are permeable to the low-molecular carbohydrates, and therefore the carbohydrates in storage organs are usually polymerized. Sometimes the polymerization goes so far that the carbohydrates become insoluble in water, e.g. starch, but in other instances the stored carbohydrates remain soluble although appreciably polymerized. The best-known soluble polysaccharide is probably inulin, which is built up exclusively of fructose molecules. Fructose seems to be a rather common unit in soluble polysaccharides. Johansson [1889] found several inulin-like carbohydrates in the Gramina. Parkin [1899] found what he called inulin (i.e. fructose-yielding polysaccharides) in the bulbs and tubers of several monocotyledons, sometimes together with starch. Monosaccharides other than fructose also build up soluble polysaccharides. Glucose, mannose and galactose are all found as constituents of polymeric soluble carbohydrates (lichenin, "Konjak"-mannan, polygalactans and others).

During an investigation of soluble high-molecular carbohydrates in plants [Svedberg & Gralén, 1938] it was found that the sap from the bulbs of different species of the family Liliaceae gave distinct and characteristic sedimentation diagrams on ultracentrifugal examination. This fact led to a more exhaustive investigation of different species and genera of Liliaceae and the closely related plant families.

MATERIAL AND ITS PREPARATION

The monograph of Engler & Prantl [1930] has been used as the reference to botanical systematics.

The work has been limited to the plant families Liliaceae, Amaryllidaceae and Iridaceae. These three families belong to the group Liliifloreae, which is divided into the subgroups Juncineae (rush plants), Liliineae (lily plants), and Iridineae (iris plants). In general the rush plants have only small dry rhizomes and, consequently, they were considered not to be suitable objects for this investigation. The Liliineae include the families Liliaceae and Amaryllidaceae together with some smaller families containing mostly tropical species. Iridaceae is the only family of Iridineae.

About 75 different species, representing a selection from the three families mentioned, have been investigated. A large proportion of the material was obtained from the Botanical Garden of Uppsala. Most of the other material was bought from Tubergen Ltd., Haarlem, Holland.

From the biological point of view it is certainly of greatest interest to study the polysaccharides in as nearly as possible the native state. The preparation was made as follows. The bulb or the tuber for investigation was peeled and grated or ground in a meat-grinder in order to break up the cell structure. The grater has been used almost exclusively. There is less risk of cracking the starch grains, which might cause solution of the inner part of the grain, the amylose. The

grated bulb was mixed with some distilled water to recover more of the dissolved material, especially if the bulb did not contain much juice. In most cases an amount of water equivalent to the weight of bulb material was added. After mixing for 2 min. with a glass rod the sap was pressed out through a filtering cloth and centrifuged free from starch and other undissolved material. In order to avoid enzymic breakdown the preparations were always made immediately before the ultracentrifuging. The time from the beginning of the preparation until the start of the centrifuge experiment usually did not exceed half an hour.

In many instances it was found that besides the high-molecular substances the juice contained low-molecular materials (salts, monosaccharides etc.) in such large amounts that they influenced the sedimentation diagram very much. To reduce this effect the solutions were dialysed for 2 days at $+4^{\circ}$ against $0.2\text{ }M$ NaCl, which was used as a standard solvent throughout the investigation. The salt concentration used is certainly sufficient to eliminate electrostatic effects, owing to charges on the sedimenting large molecules. During dialysis some precipitate often formed, but most of the high-molecular substances were left in solution, and the same diagram peaks have always been obtained from the fresh and the dialysed solutions if allowance is made for the low-molecular material in the freshly prepared solutions. The precipitate formed has not been further investigated. It is probable that the precipitation was due to decreased solubility brought about by the lowering of the temperature and of the salt concentration.

METHODS

The construction and operation of the ultracentrifuge is described by Svedberg & Pedersen [1940].

The ultracentrifuge experiments were carried out with the scale method of Lamm [1937; 1940]. The centrifugal force amounted to 300,000–350,000 times gravity at the speed used (65,000–70,000 r.p.m.). The cell thickness was 12 mm. Violet or green monochromatic light from a Hg arc lamp has been used for the photographic observations, depending on the colour of the solution. Most of the solutions were almost colourless, allowing use of the violet light. Some plant juices show a tendency to grow dark soon after the cell structure has been destroyed, owing to oxidative formation of some coloured substance by exposure to the air. It was possible to dialyse away part of the colour. Some of the remaining colour sedimented in the ultracentrifuge. The visible sedimentation column slowly grew brighter during the experiment, but a sharp colour boundary was never obtained, and it was never possible to assign the colour to any of the observed high-molecular substances (carbohydrates or proteins).

The diagrams presented here are ordinary scale diagrams from ultracentrifuge experiments. The abscissa is the distance from the centre of rotation (in mm.). To the left is the meniscus of the solution at a distance of 58–60 mm. from the centre of the rotor, and to the right is the bottom of the cell about 72 mm. from the centre. The ordinate represents the scale line displacement (in mm.), which is proportional to the concentration gradient in the cell. Each maximum of the curve represents a sedimenting boundary. The sedimenting substance is to the right of the boundary, and to the left is the pure solvent from which the sedimenting substance has disappeared. A broad peak means a diffuse boundary, a high and narrow peak means a sharp boundary. For each diagram the time after reaching full speed (in min.) and the scale distance (in mm.) are noted. The scale line displacement is proportional to the scale distance.

The continuous curves are obtained from dialysed solutions, the broken curves from the native juices. All diagrams are obtained with 0.2 *M* NaCl as reference scale. The low-molecular material in the juice always sediments a little from the meniscus, but its diffusion is so large that it does not give rise to any peak in the diagram. It is seen only as a raising of the curve towards the meniscus. The effect of the low-molecular material is clearly visible on Fig. 24 (*Narcissus poetaz*). The same diagram illustrates the fact that the sedimenting material moves faster in a dialysed solution. The low-molecular material in the juice decreased the sedimentation rate by increasing the density and viscosity of the solvent.

Colorimetric analyses for carbohydrates according to Dische [1930] have been made on practically all of the dialysed solutions investigated. Kjeldahl analyses for nitrogen have also been made. All of the solutions contained both carbohydrate and N but there were extreme variations in the quantities. The N found analytically has been taken as an indication of protein. Several of the solutions containing large amounts of N were tested by heating for 5 min. in boiling water. Coagulation always took place, and analyses of the filtrates showed that most of the N had disappeared, whereas the carbohydrate content was not changed. This suggests that the assumption that the N represented protein is roughly correct.

Table 1

Species	Sedimentation constants	Concentration in g. per 100 ml.					Nature of high-molecular material	Fig.	
		Calculations from the diagram	Carbo-hydrate	By analyses		% prot. = 6 x N			
				Nitrogen					
				N					
LILIACEAE:									
Melanthioideae:									
<i>Colchicum vernum</i>	3.3; 5.4	0.58; 2.29	0.11	—	—	Protein	(1)		
<i>C. autumnale</i>	3.3; 6.3	0.12; 0.92	0.11	0.172	1.03	Protein	(1)		
<i>C. variegatum</i>	3.1; 6.0	0.18; 1.00	0.11	0.196	1.17	Protein	1		
Allioideae:									
<i>Gagea pratensis</i>	1.1; 7.7; 12.5	0.34; 0.18; 0.78	0.56	0.184	1.10	c.h. + prot.	2		
<i>Allium ascalonicum</i>	(7.7)	0.06	0.09	0.009	0.05	—	3		
<i>A. Heldreichii</i>	1.6	0.31	0.21	—	—	c.h.	4		
<i>Brodiaea uniflora</i>	3.1	3.91	8.8?	0.184	1.10	Mostly c.h.	(14)		
Lilioideae:									
<i>Lilium candidum</i>	0.7	Not calculable	1.6	0.030	0.18	c.h.	(5, 6)		
<i>L. bulbiferum</i>	0.9	0.96	1.0	0.048	0.29	c.h.	(5, 6)		
<i>L. martagon</i>	1.3	0.25	—	—	—	c.h.	5 (6)		
<i>L. tigrinum</i>	0.8	0.46 uncertain	0.89	0.020	0.12	c.h.	(5), 6		
<i>L. speciosum</i>	1.1; 5.7	0.64; 0.39	0.56	0.067	0.40	c.h. + prot.	7		
<i>L. Henryi</i>	1.1	0.38	—	—	—	c.h.	(5, 6)		
<i>L. Maximowiczii</i> x <i>Willmottiae</i>	1.0	0.84	1.31	0.038	0.23	c.h.	(5, 6)		
<i>Fritillaria meleagris</i>	2.3; 6.2	0.73; 0.21	0.22	0.224	1.34	Mostly prot.	8		
<i>F. imperialis</i>	2.4	0.50	0.06	0.141	0.84	Mostly prot.	(8)		
<i>F. camtschaticensis</i>	1.2	0.53	0.76	0.052	0.31	c.h.	9		
<i>F. pluriflora</i>	1.1	0.25	0.28	0.018	0.11	c.h.	(14)		
<i>Tulipa praecox</i>	2.9; 6.0	0.26; 0.13	0.12	0.056	0.34	c.h. + prot.	(11)		
<i>T. Gesneriana</i>	3.4	1.10	0.44	0.180	1.08	Mostly prot.	10		
<i>T. biflora</i>	3.4; 6.4	0.39; 0.21	0.11	0.071	0.42	c.h. + prot.	11		
<i>T. sylvestris</i>	3.4; 5.7	0.17; 0.24	0.30	—	—	c.h. + prot.	12		
<i>Erythronium dens canis</i>	3.2	0.56	0.07	—	—	Protein	(10)		
<i>Calochortus albus</i>	2.9	0.11	0.03	0.012	0.08	Protein	(10)		

Table 1 (cont.)

Species	Sedimentation constants	Concentration in g. per 100 ml.				Nature of high-molecular material	Fig.
		Calculations from the diagram	Carbo-hydrate	By analyses			
				Nitrogen			
				N	% prot. = 6 × N		
Scilloideae:							
<i>Dipcadi serotinum</i>	1.3	0.45	0.61	0.013	0.08	c.h.	(14)
<i>Scilla sibirica</i>	1.1; 1.7	0.6; 2.4	3.7	0.108	0.65	c.h.	13
<i>Camassia esculenta</i>	1.9	0.88	1.04	0.020	0.12	c.h.	(14)
<i>Ornithogalum comosum</i>	2.4	0.46	0.73	0.007	0.04	c.h.	(14)
<i>O. umbellatum</i>	1.5	2.0	3.55	0.012	0.07	c.h.	(14)
<i>Chionodoxa gigantea</i>	1.2	0.46	0.58	0.020	0.12	c.h.	(14)
<i>Puschkinia scilloides</i>	1.2; 1.7	0.90; 0.24	1.39	0.042	0.25	c.h.	(13)
<i>Hyacinthus orientalis</i>	1.6	1.7	1.8	0.037	0.22	c.h.	14
<i>Muscari botryoides</i>	1.7	1.7	2.06	0.005	0.03	c.h.	(14)
<i>M. comosum</i>	3.2	0.92	1.62	0.021	0.13	c.h.	(14)
Asparagoideae:							
<i>Polygonatum multiflorum</i>	1.3	2.75	2.95	0.014	0.09	c.h.	(14)
<i>Paris quadrifolia</i>	2.8	0.09	0.22	0.015	0.09	?	(5, 6)
AMARYLLIDACEAE:							
Amaryllidoideae:							
<i>Galanthus nivalis</i>	4.3	0.14	0.16	0.032	0.19	?	(10)
<i>G. Elwesii</i>	1.2	0.46	0.78	0.014	0.09	c.h.	15
<i>Leucojum vernum</i>	1.3; 4.3	0.71; 0.52	0.45	0.126	0.76	c.h. + prot.	16, 17
<i>L. aestivum</i>	1.6; 3.6	0.20; 0.19	0.22	0.042	0.25	c.h. + prot.	(16)
<i>Amaryllis Belladonna</i>	—	—	0.04	0.008	0.05	?	(3)
<i>Crinum Moorei</i>	1.0	0.16	1.1?	0.009	0.06	?	(3)
<i>Ixiolirion Ledebouri</i>	1.2; 12.2	0.32; 0.53	0.61	0.079	0.47	c.h. + prot.	18
<i>Lycoris radiata</i>	1.0; 3.6	0.13; 0.09	0.22	0.048	0.29	c.h. + prot.	19, 20
<i>Narcissus pseudonarcissus</i>	1.4	0.50	0.47	0.018	0.12	c.h.	(5, 6)
<i>N. cyclamineus</i>	1.1	1.01	1.11	0.051	0.31	c.h.	(14)
<i>N. juncifolius</i>	1.3	0.55	0.64	0.030	0.18	c.h.	(5, 6)
<i>N. jonquilla</i>	2.7; 4.0	0.42; 0.99	0.21	0.207	1.24	c.h. + prot.	21
<i>N. tazetta</i>	1.8	0.69	0.37	0.087	0.52	c.h. + prot.	22
<i>N. poeticus</i>	1.6; 1.8	0.10	0.11	0.008	0.05	c.h. + prot.	23
<i>N. poetaz</i>	1.9	0.34	0.30	0.019	0.11	c.h. + prot.	24
<i>N. Bulbocodium</i>	1.2	0.37	0.36	0.018	0.11	c.h.	(5, 6)
<i>N. incomparabilis</i>	1.4; 2.4	0.56; 0.48	0.56	0.073	0.45	c.h. + prot.	(7)
<i>N. Leedsii</i>	1.2	0.25	0.30	0.039	0.23	c.h. (+ prot.)	(5, 6)
Hypoxidoideae:							
<i>Alstroemeria aurantiaca</i>	—	—	0.16	0.013	0.08	?	(3)
IRIDACEAE:							
<i>Crocus reticulatus</i>	3.1; 6.2	0.39; 0.20	0.07	0.094	0.56	(c.h.) + prot.	25
<i>C. sativus</i>	2.7; 5.9	0.36; 1.74	0.68	0.249	1.49	c.h. + prot.	26
<i>C. speciosus</i>	3.0; 6.5	0.35; 0.24	—	—	—	(c.h.) + prot.	(25)
<i>Freesia refracta</i>	3.1	2.30	0.20	0.287	1.72	Protein	(10)
<i>Sparaxis tricolor</i>	2.9; 5.4; 17.8	1.14; 0.66; 1.38	0.64	0.484	2.90	c.h. + prot.	27
<i>Gladiolus segetum</i>	2.9; 6.5	0.73; 0.92	0.16	0.161	0.97	Mostly prot.	(28)
<i>G. communis</i>	2.8; 6.5	0.50; 0.77	—	—	—	Mostly prot.	28
<i>G. byzantinus</i>	2.9; 6.9	1.02; 0.23	0.16	0.169	1.01	Mostly prot.	29
<i>G. grandis</i>	3.2; 6.9	0.58; 0.60	0.07	0.138	0.83	Mostly prot.	(28)
<i>G. cuspidatus</i>	3.2; 6.7	1.09; 0.41	0.08	0.210	1.26	Mostly prot.	(29)
<i>G. anatolicus</i>	2.7	0.31	0.02	0.061	0.36	Protein	(17)
<i>G. Colvillei roseus</i>	3.0; 6.3	0.84; 1.11	0.15	0.195	1.17	Mostly prot.	(28)
<i>Moraea tricuspidis lutea</i>	4.5	2.9	0.27	0.307	1.84	Protein	(10)
<i>Iris squalens</i>	8.0	0.02	0.02	0.009	0.05	?	(3)
<i>I. reticulata</i>	0.9; 8.7	0.72; 0.59	1.14	0.152	0.91	c.h. + prot.	30

13. *Scilla sibirica* 14. *Hyacinthus orientalis* 15. *Galanthus Elwesii* 16. *Leucojum vernum* 17. *Leucojum vernum*, heated 18. *Ixiolirion Ledebouri* 19. *Lycoris radiata* 20. *Lycoris radiata*
(light absorption method) 21. *Narcissus jonquilla* 22. *Narcissus tazetta* 23. *Narcissus poeticus* 24. *Narcissus poetax*

The concentration of the sedimenting material has been calculated from the sedimentation diagrams, assuming a refractive index increment dn/dc of 1.5×10^{-3} , which is an approximate value for carbohydrates (cf. p. 246). The comparison of the analyses and the calculations from the curves very often gives information about the nature of the sedimenting material. The method is, however, not very accurate. It was very often not possible to remove all turbidity from the solutions used for analyses. This would give too high analytical values, because in the ultracentrifuge the turbidity always disappeared at a relatively low speed (15,000–20,000 r.p.m.). Sometimes it was difficult to determine the exact

position of the base line of the sedimenting curves, especially when the peak was broad and even elongated over the whole cell (see, for instance, Fig. 14, *Hyacinthus orientalis*). The base line position affects the concentration calculation to a considerable extent. In spite of the difficulties mentioned, the procedure has given good results in many instances.

For every species at least two ultracentrifuge experiments have been made, one on the freshly prepared juice, one on the dialysed solution. The results are collected in Table I and in Figs. 1–30. The Table contains the sedimentation constants¹ (s_{20}) obtained from the dialysed solutions, reduced to pure water and the temperature 20°. The concentration calculations from the diagrams are given in the next column. If several peaks are obtained in the same diagram, the sedimentation constants are given in order starting with the lowest, and the concentrations for the corresponding peaks in the same order. The results of the analyses for carbohydrate and N are also reported. The protein content has been calculated, assuming it to be 6 times the N content. In one column is reported the nature of the sedimenting substance, as indicated by the analysis. The last column gives a reference to the number of the corresponding diagram. If the number is put in parenthesis, the diagram referred to was obtained not from the species in question but from another of the same sedimentation type.

¹ Sedimentation constants are given in units of 10^{-13} .

DISCUSSION OF RESULTS

Only a few species failed to yield a noticeable amount of dissolved high-molecular material in the bulb juice. *Allium ascalonicum*, the cultivated onion, had considerable low-molecular material in the juice, but after dialysis practically nothing was visible (Fig. 3). Another species of the genus *Allium*, *A. Heldreichii*, yielded a small amount of a high-molecular carbohydrate (Fig. 4). This raises the question as to whether the cultivation has caused the breakdown of a pre-existent polysaccharide in *A. ascalonicum*.

There is an analogy in the genus *Tulipa*. The cultivated tulip, *Tulipa Gesneriana*, contains a large amount of low-molecular material hiding possible peaks. After dialysis, however, a large peak appears (Fig. 10). The analyses show a high N content but only a small amount of carbohydrate, and therefore it is probable that the peak represents a protein. The same diagram has been obtained from different varieties of *T. Gesneriana*. The wild tulips here investigated, *T. sylvestris*, *T. praecox*, *T. biflora*, all show two peaks (Figs. 11, 12). Precipitation by alcohol and redissolving (which was not complete) gave a solution which was practically N-free. The sedimentation diagram of that solution showed absence of the faster moving peak of the original diagram. Obviously the protein has been denatured by the alcohol, and, consequently, the carbohydrate of these species belongs to the slower component. The alcohol-precipitate from *T. Gesneriana* could not be redissolved in water in any appreciable amount.

The *T. Gesneriana* type diagram with a large protein constituent was found with some of its near relatives, *Erythronium dens canis* and *Calochortus albus*. Other, more distantly related plants, also had a high protein content and gave similar diagrams. This was found for *Colchicum* (Fig. 1, fam. Liliaceae), *Freesia* and *Moraea* (fam. Iridaceae). For *Colchicum* and *Moraea*, however, the sedimentation constants were higher.

Where there is only one peak in the diagram, it is generally rather easy to decide from the analyses whether it is caused by a carbohydrate or a protein. If two or more peaks appear and the analyses show presence of both N and carbohydrate, the question of identification of the substances with the peaks of the diagram arises. It is sometimes possible to draw conclusions from the relative proportions of the peaks. It is obvious that the largest peak in *Lilium speciosum* (Fig. 7) represents a carbohydrate and the smaller peak, which sediments more rapidly, is a protein. In *Crocus sativus* (Fig. 26) the faster, large peak is the protein, while the low concentration of high-molecular carbohydrate appears in the diagram as the smaller, slow peak.

If the difference between the concentrations is not so large, however, the method is not accurate enough to give any sure results. The precipitation with alcohol gave results for the *Tulipa* species. Another method that was used successfully was the heating of the solutions for 5 min. in a boiling water bath. In most cases the protein coagulated but the carbohydrate was left in solution. The ultracentrifuging of the remaining solution always showed that one of the peaks disappeared as a result of the heating. The two diagrams of *Leucojum vernum* (Figs. 16 and 17) were obtained before and after heating. The same procedure has been applied to *Narcissus incomparabilis*, *Crocus sativus* (Fig. 26), *Gladiolus byzantinus* (Fig. 29) and *Iris reticulata* (Fig. 30), and the results always showed that the faster-sedimenting peak represented a protein, whereas the carbohydrate was slower. In the case of *Gladiolus byzantinus*, the slower peak also contained protein as well as the carbohydrate, this being shown by a

marked decrease in that peak after heating, whereas the faster peak disappeared completely.

Ultracentrifuging in ultraviolet light is probably the mildest possible treatment which can be given the solution and be expected to yield information about the nature of the sedimenting substances. The proteins have a strong light absorption band around 260 $m\mu$, whereas the carbohydrates do not absorb until at a very much lower wave-length. Chlorine and bromine filters have been used for obtaining a suitable wave-length region for the protein absorption band [Svedberg & Nichols, 1926]. The applicability of the method is shown by the example of *Lycoris radiata* (fam. Amaryllidaceae). The first diagram (Fig. 19) is the ordinary scale diagram. The second one (Fig. 20) shows the photographic blackening curves from the ultraviolet light absorption experiment. The sedimentation constants calculated from the former are 1.0 and 3.6. The latter gives $s_{20}=3.3$ which is in good agreement with the faster peak in the scale diagram, while the slower peak cannot be detected in the absorption diagram and so represents the polysaccharide.

Other methods for separating proteins and carbohydrates have not been used, but there are certainly several mild methods that could be applied. Electrodialysis would probably precipitate the proteins which presumably are mainly globulins, while the carbohydrates would stay in solution. Separation centrifuging [Tiselius *et al.* 1937] could possibly be used if the sedimentation constants are widely different. Electrophoresis [Tiselius, 1937; 1938] would presumably give separation at a suitable pH . The proteins are certainly carriers of electrical charges, but the carbohydrates are more likely non-electrolytes [cf. Seibert *et al.* 1938].

In some cases it has not been possible to decide whether the peaks are caused by proteins or carbohydrates. The diagram of *Gagea pratensis* (Fig. 2), for instance, has no less than three peaks, one of them with the very high $s_{20}=12.5$. It is noticeable that *Gagea* yields such a detailed diagram while *Allium*, very closely related systematically, gives no indication of high-molecular substances.

The very high sedimentation constant (~ 12) of the fastest *Gagea*-peak is found also with *Ixiolirion Ledebouri* (Fig. 18, fam. Amaryllidaceae). It is very characteristic for both these species although they are systematically widely different. A still higher sedimentation constant, $s_{20}=17.8$, was obtained with *Sparaxis tricolor* (Fig. 27, fam. Iridaceae), which contains at least three different high-molecular constituents, as indicated by the dotted lines in the diagram. Heating gave no information about *Ixiolirion* or *Sparaxis*, because the proteins did not precipitate completely.

It seems impossible to draw parallels between the centrifuge diagrams and the larger divisions of systematics. Similar diagrams are found with material from different families and widely different diagrams are obtained with material from the same family, showing that the carbohydrate storage is not similarly organized within the same family. In the smaller units, the genera, the diagrams are, however, usually very similar. There are exceptions even to this rule, and they sometimes have a special interest.

Fritillaria meleagris and *F. imperialis* yield similar diagrams (Fig. 8) and contain almost exclusively proteins as the high-molecular constituents of their bulb-juices. *F. camtschaticensis* yields a different diagram (Fig. 9), a very sharp, slowly moving peak, representing a carbohydrate. The same picture is given by almost all *Lilium* species that have been examined (Figs. 5, 6). It is known that *F. camtschaticensis* is more closely related to the genus *Lilium* than are the other *Fritillaria* species [Buxbaum, 1937]. (Engler & Prantl [1930] assign it to a sub-

genus called *Liliorhiza*.) On the basis of the present results it would even be preferable to assign it to the genus *Lilium* itself.

The subfamily Scilloideae of Lilioideae yielded a typical diagram. It was obtained from the genera *Dipcadi*, *Camassia*, *Ornithogalum*, *Chionodoxa*, *Hyacinthus* and *Muscari* (Fig. 14). It shows a wide peak that spreads out more and more during the sedimentation. The sedimentation constant varied considerably for different species, but it is possible that most of the variation was due to differences in concentration which affect the sedimentation rate. The analyses showed that the sedimenting material was a carbohydrate.

An unusual diagram was obtained from *Scilla sibirica* (Fig. 13). It showed two peaks, both of which were from carbohydrates judging by the analyses. Heating the solution to 100° did not change the character of the diagram, even though some precipitation occurred which lowered the N content. It is possible to resolve the diagram into its two constituents as indicated on the diagram. The faster-moving peak is broad and of the same type as those in the diagrams from the other Scilloideae. The slower peak has a different appearance, being sharper and much higher, although the concentration, proportional to the area of the peak, is lower than for the more rapidly moving carbohydrate. The sharp peak is very similar to those obtained from the *Lilium* bulbs (Figs. 5, 6).

The diagram from *Puschkinia scilloides* was similar to that of *Scilla sibirica*. It is obvious that the external similarities between the two species, which have given the *Puschkinia* species its name, correspond to an internal resemblance in the physico-chemical organization of the plants.

Carbohydrates of the same type as those of the genus *Lilium*, resulting in the very sharp boundary in the sedimentation diagram (Figs. 5, 6), are found also in the family Amaryllidaceae within the genus *Narcissus*. All the juices giving this diagram are very slimy. There are also slimy bulbs, however, which give different diagrams (*Hyacinthus* and others, especially in the subfamily Scilloideae).

The genus *Narcissus* shows different types of composition of the high-molecular substances in the bulb juices. The "*Lilium*-peak" (Figs. 5, 6) is obtained from *N. pseudonarcissus*, *N. juncifolius*, *N. Bulbocodium* and *N. Leedsi*. *N. incomparabilis* also shows it but combined with a faster-sedimenting substance which has proved to be a protein (precipitates on heating). *N. cyclamineus* yields the *Hyacinthus*-diagram (Fig. 14). *N. jonquilla* contains considerable protein, probably in both the components of the diagram (Fig. 21). *N. tazetta* has the very sharp *Lilium*-peak (Fig. 22). *N. poeticus* contains much low-molecular material, and the high-molecular substances are not visible in the diagram until after dialysis (Fig. 23). It gives two small, rather diffuse peaks. *N. poetaz* (Fig. 24), a hybrid *N. poeticus* × *N. tazetta*, has a large amount of low-molecular material like *N. poeticus*, but the sharp carbohydrate-peak of *N. tazetta* is distinct. Obviously it has acquired properties in this respect from both its parents. The ultracentrifuge method here opens a new field for genetics, and it will be of great interest to investigate the way in which the properties of reserve-carbohydrate storage are inherited. Some research in this direction has been started.

Most of the soluble carbohydrates found in bulb juices can be arranged into two classes as regards their sedimentation behaviour. The first one is typically represented by the *Hyacinthus* (Fig. 14) and the second one by the *Lilium* bulbs (Figs. 5, 6). The *Hyacinthus* polysaccharide gives a very broad peak, spreading almost over the whole sedimentation column, while the *Lilium* polysaccharide

sediments with a very sharp boundary. The sedimentation constants for both are of the same order of magnitude, 1–2, but the differences in the sedimentation behaviour indicate quite different physico-chemical properties.

The *Hyacinthus* type most probably represents a mixture of different molecular sizes, varying continuously according to some distribution law. The limits are presumably rather wide, as judged by the sedimentation curves. The hydrolysis products of the carbohydrates have not been examined, but it is probable that these polysaccharides are in some instances identical with the polyfructans found earlier especially in the subfamily Scilloideae of the family Liliaceae [Parkin, 1899]. Parkin pointed out that most species of this subfamily contain "inulin" together with starch. It should be noted that the inulin in the juices pressed from the tubers of *Inula helenium*, *Dahlia coccinea* and *Helianthus tuberosus* (all belonging to the family Compositae), gave sedimentation diagrams of the same type and a sedimentation constant of the same magnitude.

The second type of polysaccharide, the *Lilium*-type, is of more interest because of its peculiar sedimentation diagram. It shows, as mentioned, a very sharp boundary for the sedimenting material, indicated by a high and sharp peak in the diagram. A protein or other globular molecule with the same low sedimentation constant would have so high a diffusion that no maximum in the curve would be obtained, because it would never leave the meniscus at the same centrifugal force. In this case, however, there is scarcely any diffusion indicated in the centrifuge cell. The same phenomenon has been observed by earlier investigators on other substances, especially cellulose derivatives [Kraemer & Nichols, 1940; Signer, 1940]. The sharpness of the peak indicates a thread-like shape of the molecules. These molecules are considerably hindered in their motion by each other, and the effect is greater the higher the concentration. The sedimentation constant is therefore higher in a more dilute solution. If some molecules are left behind, they, being in a more dilute solution, sediment at a more rapid rate. On the opposite side of the boundary where the concentration increases, the molecules will move more slowly. These effects counteract the diffusion which would normally be expected, and cause the sharpening of the boundary.

Purifying the polysaccharide of *Lilium speciosum* by precipitation with alcohol and redissolving in water did not change the sedimentation diagram or the sedimentation constant for a given concentration. The relation between the concentration and the sedimentation constant is given by Fig. 31. A diffusion experiment was carried out on the purified material according to Lamm's method [1937]; cf. Polson [1939]. Using the formula

$$D = \frac{x_1^2 - x_2^2}{4t \log_e y_2/y_1}$$

where D is the diffusion constant, t the time, x the distance from the original boundary and y the height of the concentration gradient curve at the point x , it is possible to calculate diffusion constants from different parts of the curves obtained. D showed a drift along the curve, which proves inhomogeneity. From the sedimentation experiment one would expect the carbohydrate to be homogeneous, i.e. contain molecules of only one size, but the diffusion experiment shows that such a conclusion is not justified. Near the original boundary the lowest values of the diffusion constant are obtained because the large molecules diffuse more slowly. With increasing distance from the original boundary the diffusion constants increase, because the curve is mainly determined by the smaller molecules there. The drift in D for the *Lilium* polysaccharide was from

about 4.0 to 5.4.¹ This means a still greater variation in the true diffusion constant, because the smallest value here given was obtained from the highest part of the curve where both the larger and the smaller molecules are effective in determining the gradient, while the largest D was obtained from the low part where the curve is almost exclusively determined by the smaller molecules. For the molecular weight a mean value of 20,000 was obtained, assuming the partial specific volume to be 0.64 and using the formula

$$M = \frac{RTs}{D(1 - V\rho)}$$

[Svedberg & Pedersen, 1940], where M is the molecular weight, R is the gas constant, T the absolute temperature, s the sedimentation constant (taken at zero concentration), D the diffusion constant, V the partial specific volume and ρ the density of the solvent (water). The value 20,000 corresponds to about 125 units of monosaccharides.

Fig. 31. Relation between concentration and sedimentation constant of purified carbohydrates from *Lilium speciosum* and *Narcissus Leedsi*.

Another polysaccharide with the same sedimentation behaviour was prepared from the bulbs of *Narcissus Leedsi* Southern Gem (from Tubergen Ltd.). The water-extract from the bulbs was precipitated twice by alcohol, the redissolved polysaccharide was filtered through filter paper pulp, dialysed and electro-dialysed. Kjeldahl analyses showed that the N content was less than 1 % of the dry material. The sedimentation constant depends on the concentration as shown by Fig. 31. The polysaccharide was very stable; it could be heated to 100° for 30 min. in neutral solution, or dried *in vacuo* over P_2O_5 and redissolved without any change of s_{20} . In the dry state it was a transparent, fairly brittle film, while the solution was faintly opalescent. The partial specific volume in the solution was 0.640, which is appreciably lower than the figures for proteins (in general 0.73–0.75 [Svedberg & Pedersen, 1940], but in good agreement with

¹ Diffusion constants are given in units of 10^{-7} .

those for cellulose (0.64 [Stamm, 1930]). The refractive index increment dn/dc (c in %) was 1.46×10^{-3} , which corresponds closely to the values for other carbohydrates, both mono- and poly-saccharides. The specific rotatory power was $\alpha_D = -42^\circ$, which is of the same order of magnitude as for inulin (-39° , -40°). The polysaccharide was hydrolysed by boiling for 2 hr. in 4% H_2SO_4 , and a preliminary study of the hydrolysis products was made. Seliwanoff's reaction [Roe, 1934] with resorcinol showed that only about 20% of the material was a ketose (probably fructose). Mannose could be demonstrated in a small amount ($\sim 10\%$) by its phenylhydrazone (M.P. 190°). The rest of the carbohydrate (70%) was probably glucose, because it yielded a large amount of phenylglucosazone (M.P. 204°).

Diffusion experiments have shown that the *Narcissus Leedsii* polysaccharide was polydisperse, the variation of D being from about 2 at the top of the curve to about 4 at the bottom. No dependence on concentration could be demonstrated. A mean value of 50,000 was obtained for the molecular weight, if calculated with the sedimentation constant at zero concentration, 1.9. Viscosity measurements gave $\eta_{sp}/c = 47$, where c is expressed in base molarity, according to Staudinger [1932]. This would give a Staudinger proportionality factor $K_m = 9 \times 10^{-4}$, which is comparatively high and gives still more evidence of the thread-like shape of the molecules.

No completely homogeneous substance has been found among the polysaccharides investigated. Nature seems to prefer a mixture of polymeric homologues, even if the polydispersity is definitely different for different systems. For some of the natural soluble products the heterogeneity is less than for artificial solutions of polysaccharides such as cellulose and starch and for synthetic products like polystyrenes. The reason for the polydispersity of polymeric carbohydrates is possibly that the differences between a polysaccharide molecule and its nearest higher and lower homologues are very small. The elongation of the chain with one or a few units will not change the properties of the molecule much, particularly as the largest part of the molecule is far away from the new linkage. Another class of high-molecular compounds, the globular proteins, have a different type of organization. They consist of different amino-acids in regular proportions, the molecules have a more spherical shape, and there is a strict organization within each molecule. Therefore the molecule must be totally built up according to its plan before it is suited to fill its function in the vital processes. When it is fully completed, it has no use and no place for any more amino-acids, and so all the protein molecules of the same kind are equal in size and shape. If a foreign amino-acid were linked to the complete protein, it would change the organization and shape of the molecule more than a monosaccharide would alter the polysaccharide molecule to which it became attached. The polysaccharide is suitable for its functions even if it consists of relatively few monosaccharide units. Its constituents are more homogeneous than the widely different amino-acids of the protein. Very often the monosaccharide units are the same through the whole molecule, and even if they are not, the differences among the hexoses are not large. Therefore the polysaccharide can be built up continuously without marked changes in the qualitative properties of the molecule. There is certainly some regulation of the size of the molecules, probably of enzymic nature, but it need not definitely restrict the number of monosaccharide units in every molecule, and, consequently, there is a variation within wide or narrow limits.

From the biological point of view it would be of great interest to know if the soluble polysaccharides constitute any transition form between the solid poly-

saccharides (starch, cellulose etc.) and their building units, the monosaccharides. Certainly some of the carbohydrates studied here are reserve materials in the plants and so have some function of their own, but it is very possible that others serve only as an intermediate stage in the breakdown of starch or other reserve-carbohydrates for transport in the plants or in the building up of the solid, structural polysaccharides, the prototype of which is cellulose. Further investigations are proceeding to give a more detailed outline of the physico-chemical properties of some of the soluble polysaccharides found in plant juices. It is hoped that this research will contribute to the solution of the question of the functions of these substances in the plants.

SUMMARY

1. An ultracentrifugal study of the water-soluble high-molecular materials contained in the bulb juices of about 75 species of the families Liliaceae, Amaryllidaceae and Iridaceae has been made.

2. The different species yield widely different sedimentation diagrams. They contain proteins and polysaccharides of different properties and in different proportions.

3. There is generally a pronounced similarity among the species of the same genus with regard to the content of high-molecular material. The seven *Lilium* species investigated, for instance, all contain a large amount of dissolved polymeric carbohydrate, and they all give the same type of sedimentation diagram. Outside the genera, the differences are large even within the same family. Species of *Fritillaria* and *Tulipa*, which belong to the same subfamily as *Lilium*, generally contain in solution more protein than carbohydrate and yield diagrams widely different from the *Lilium* diagrams. One *Fritillaria* species, however, *F. camtschaticensis*, which is closely related to *Lilium*, gives a diagram of the *Lilium* type.

4. Among the carbohydrates found there are two classes, distinguished by their sedimentation behaviour. One of them, the *Hyacinthus* type ($s_{20} \sim 1-2$), found especially within the subfamily Scilloideae of the family Liliaceae, gives a broad, diffuse boundary, indicating a marked polydispersity. The second one, the *Lilium* type, obtained with the genera *Lilium* (Liliaceae) and *Narcissus* (Amaryllidaceae), although having a sedimentation constant of the same magnitude, yields a very sharp boundary, which is probably due to the thread-like shape of the molecules.

5. Two individuals of the *Lilium* type polysaccharide, from the species *Lilium speciosum* and *Narcissus Leedsii*, have been purified and subjected to a more detailed physico-chemical investigation. The sedimentation constants extrapolated to zero concentration were found to be, for the *Lilium* species 1.6, and for the *Narcissus* species 1.9. Diffusion experiments in combination with these figures gave mean molecular weights of 20,000 and 50,000 respectively.

6. The polydispersity of the soluble polysaccharides has been discussed in comparison with the monodispersity of the globular proteins.

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