when chromatogram solvents are used that are no more acid than the phenol solvent. These findings indicate that the radioactive phosphate in the FAD area is a contamination, rather than part of a chemical entity.

SUMMARY

1. Paper chromatographic procedures for the separation of flavin nucleotides from other phosphates are described.

2. The incorporation of [^{32}P] phosphate into flavin mononucleotide and flavin-adenine dinucleotide (FAD) in liver suspensions is negligible under conditions where ATP rapidly becomes radioactive. It is concluded that the phosphate of free flavin nucleotides plays no direct part in oxidative phosphorylation.

3. Evidence is presented which indicates that the contrary findings of Hummel & Lindberg (who reported a rapid incorporation of [^{32}P] phosphate into FAD) can be explained by incomplete separation of FAD from other phosphates by certain techniques. Contamination of FAD with other phosphates occurs when FAD is extracted with

phenol from aqueous, saturated, ammonium sulphate solutions and phenol-containing solvents are used for the development of paper chromatograms.

4. FAD (10^{-5} – 10^{-4}M) inhibits the oxygen uptake of liver suspensions.

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The Carbohydrates of *Agave vera cruz* Mill.

2. DISTRIBUTION IN THE STEM AND POLE

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The carbohydrate gradient in the stem of *Agave vera cruz* was previously reported upon by Srinivasan & Bhatia (1953). It was then indicated that where the carbohydrate content was high and uniform throughout the stem, such a stem belonged to a fairly aged plant. To confirm these indications, a plant which had poled was selected for study, since it is known that such a plant is mature. Data relating to the make-up and distribution of carbohydrates in the stem and the pole are presented here. Evidence of identification of the simple sugars present is given by Bhatia, Satyanarayana & Srinivasan (1953*a*). The present study was of added interest from the point of view of the production of fructose from this

source, an aspect investigated by us and for which it was desirable to have information regarding the site of maximum fructosan concentration in the plant.

EXPERIMENTAL

Materials. A plant which had infloresced and partly bulbeled was chosen and sectioned into stem and pole (inflorescence axis). These were arbitrarily further subsectioned as shown in the diagram (Fig. 1). The material from each section was grated and juice was pressed out. The total time taken for these operations was about 90 min. (normally, no significant enzymic degradation of polysaccharides takes place in this material within this period). The juice was then clarified as described previously (Srinivasan & Bhatia, 1953) and used for analysis.

Fig. 1. Stem and pole of *Agave vera cruz* drawn to scale (1 cm. = 10 in.). Shaded sections taken for experiment.

Table 1. Analysis of carbohydrates in the stem and pole of the Agave plant

(Experimental material: expressed juice from the different sections; α_D^{25} = optical rotation in 1 dm. tube; TRS = total reducing sugar.)														
Sections (see Fig. 1)	...	...	...	...	...	S_1	S_2	S_3	P_1	P_2	P_3	P_4	P_5	B
Before hydrolysis:														
Solids in the original juice (% w/v)						26.1	25.6	26.7	23.2	11.1	9.1	8.0	8.2	12.8
α_D^{25} (°)						-5.90	-6.35	-6.85	-3.03	+0.20	+0.33	-0.03	-0.30	-0.45
TRS expressed as 'fructose' (% w/v)						2.4	2.7	3.4	1.8	4.1	5.9	5.5	5.6	3.3
Material oxidizable by hypoiodite, expressed as 'glucose' (% w/v)						1.2	1.1	1.2	1.5	3.0	3.7	3.6	3.6	3.4
After hydrolysis:														
TRS expressed as 'fructose' (% w/v)						26.3	26.3	26.8	22.6	9.6	7.5	6.7	6.0	9.8
Material oxidizable by hypoiodite, expressed as 'glucose' (% w/v)						2.4	2.4	2.4	2.5	4.2	4.2	3.8	3.8	4.3
α_D^{25} (°)						-18.10	-18.30	-18.80	-13.6	-2.95	-0.65	-0.72	-0.75	-3.70

Table 2. Carbohydrate make-up in the stem and pole of the Agave plant

(Analysis of component carbohydrates separated by paper chromatographic fractionation of 0.1 ml. juice expressed from the sections. TRS = total reducing sugar. Columns a = mg. TRS in each component expressed as 'fructose', columns b = % of each component in terms of combined TRS recovered from the chromatogram.)

Sections (see Fig. 1) ...	...	S_1		S_2		S_3		P_1		P_2		P_3		P_4		P_5		B	
Carbohydrates separated on the chromatogram ...	...	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Fructose	...	0.7	2.4	1.7	5.6	2.4	8.6	0.7	3.2	1.7	16.4	2.3	37.2	2.3	36.5	2.4	35.5	0.6	5.3
Glucose	...	0.5	1.8	0.5	1.7	0.5	1.7	0.9	4.1	2.7	26.6	2.8	44.2	2.8	43.7	3.3	48.7	2.3	21.8
Sucrose	...	1.6	5.8	2.0	6.7	2.0	7.2	1.8	7.7	1.5	14.6	1.2	18.6	1.3	19.8	1.1	15.8	0.7	6.9
Spot X	...	1.0	3.7	1.4	4.5	1.0	3.7	1.4	6.1	1.0	10.2	Nil	—	Nil	—	Nil	—	0.9	8.5
Spot Y	...	2.9	10.3	3.6	11.9	2.9	10.4	4.0	17.5	3.3	32.2	Nil	—	Nil	—	Nil	—	2.2	20.8
Spot Z	...	20.9	75.8	20.9	69.7	19.1	68.5	13.9	61.3	Nil	—	Nil	—	Nil	—	Nil	—	3.9	36.6
TRS recovered (mg.)	...	27.6	—	30.1	—	27.9	—	22.7	—	10.2	—	6.3	—	6.4	—	6.8	—	10.6	—
Total fructose (mg./0.1 ml.)*	...	24.9	—	26.3	—	26.4	—	19.2	—	6.2	—	—	—	—	—	—	—	7.2	—
(Total fructose/TRS) × 100	...	90.0	—	85.0	—	94.6	—	84.6	—	60.8	—	—	—	—	—	—	—	67.9	—

* Determined colorimetrically in the original juice.

Chromatographic methods. The details have been described by Srinivasan & Bhatia (1953). The juice (0.1 ml. from a 1 ml. graduated pipette) was applied as a streak about 4 cm. long on the starting line of the paper. This was done in triplicate, leaving 5 cm. spaces between the streaks. The chromatogram was developed for 9 days by an ascending flow of the solvent (*n*-butanol:acetic acid:water, 4:1:5, v/v). The separated spots from one streak were located with benzidine trichloroacetic acid; corresponding areas were cut out from the remaining two unsprayed chromatograms. Equivalent empty areas on the paper were used as blanks. The determined blank values, corrected for areas, were deducted from the corresponding experimental results. For 50 cm.² of the paper, the average blank value was equivalent to 0.06 ml. of 0.01% thiosulphate by the ferricyanide method of Sobotka & Reiner (1930).

Methods for the determination of carbohydrates. In the original juice total soluble solids (referred to as 'solids'), total reducing sugars (TRS; expressed as 'fructose'), and aldoses (expressed as 'glucose'), before and after hydrolysis, were determined as described by Srinivasan & Bhatia (1953). Total fructose was determined in the juice, after adequate dilution and with standard fructose for comparison, by the method of Bacon & Bell (1948), using the Klett-Summerson photo-electric colorimeter (filter no. 42). The cut areas of papers carrying the separated carbohydrates and the corresponding blank papers were transferred to wide-mouthed, stoppered bottles, and known volumes of distilled water were added. The average areas of spots Z, Y and X and sucrose, glucose and fructose were approximately 50, 50, 45, 45 and 45 cm.², respectively. The volumes of water used for extraction of these spots were 50, 50, 50, 25, 25 and 25 ml., respectively. The bottles were shaken from time to time during the course of 1 hr. and the contents were filtered to free from fibre. In 10 ml. of the filtrate, without hydrolysis in the case of fructose and glucose and after hydrolysis in the others, TRS was determined by the method evolved by Hagedorn & Jensen, and then Hanes, as modified by Sobotka & Reiner (1930) and expressed as 'fructose'. Analyses carried out on duplicate spots showed an average variation of 3% between them. Results given in Table 2 are the average of duplicates and are corrected for pipette error and paper blanks.

RESULTS

The total carbohydrates, expressed as reducing sugars in terms of fructose, recovered from the chromatogram of fructosan-rich sections like the stem, exceed the TRS in the hydrolysate of the corresponding original juices, even after allowing for pipette error and paper blanks. This discrepancy is at a minimum in the case of the sections above the stem. While the results reported are by no means absolute, they could be taken as indicative of the overall distribution and make-up of the carbohydrates in the different sections of the plant.

DISCUSSION

The results obtained relate to a fully matured plant. It is found that in such a plant fructosans are most highly concentrated in the stem and are uniformly

distributed in this section. Considering the stem as a whole, solids and the TRS after hydrolysis are very nearly equal (Table 1), indicating that the total solids in the stem are entirely carbohydrate in nature. Of these, fructosans (spot Z) form about 70%, oligosaccharides (spots X and Y) about 14% while sucrose, glucose, and fructose, together form about 15%. Glucose is in the lowest concentration. The total fructose-containing residues amount to 90% of total carbohydrates, as determined colorimetrically (Table 2).

In P_1 , the bottom section of the pole, a tendency for a decrease in the fructosan concentration and an increase in oligosaccharides becomes noticeable. This tendency is accentuated in P_2 , a section further up the base of the pole, wherein fructosan is entirely absent. This leads to the assumption that the sections forming the base of the pole and below there must exist an enzyme system capable of hydrolysing the reserve carbohydrates into simple sugars for transport upwards. Such an enzyme system has indeed been observed in the plant and is now under study.

The pole has no leaves, and because of restricted photosynthesis in this region, it may well depend on carbohydrates for its metabolic needs mainly from the stem by translocation. In fact, fructosans, glucose and sucrose are the only carbohydrates in the major portion of the pole, i.e. between sections P_2 and B. In contrast, the upper extremity of the pole (section B, where the bulbels had begun to form) contained fructosans and intermediate-like those present in the stem, but in relatively lower concentrations. This suggests that the most section of the pole is one of the sites of enzymic synthesis of fructosan. That the pole contains a transfructosidase type of enzyme has been shown (Bhatia & Srinivasan, 1953).

It is noticeable that while in the stem fructosans are the major reducing sugar, in section B it is glucose. Sucrose, however, does not seem to vary significantly from section to section. Preliminary results obtained from the leaves show that the qualitative make-up of the carbohydrates in the base of the leaf where it is attached to the stem, is similar to that of the stem carbohydrates, while fructosans are absent in the top sections of the leaf (Bhatia, Satyanarayana & Srinivasan, 1953b).

SUMMARY

1. The make-up of carbohydrates of the stem and pole of *Agave vera cruz* has been determined quantitatively with the aid of paper chromatography.

2. As previously reported, fructosans form the major fraction and are most highly concentrated in the stem.

3. The base of the pole has a carbohydrate makeup exactly analogous to that of its lower contiguous section, the stem. Moving upwards along the pole, the picture changes sequentially: first fructosans disappear, then their intermediates, leaving only fructose, glucose, and sucrose. On the other hand, in the top-most section of the pole, the seat of flower-head and bulbel formation, the fructosans and their intermediates reappear; the entire picture is in fact similar to that of the carbohydrates in the stem.

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Sedimentation and Diffusion of Human Albumins

3. VARIOUS NORMAL HUMAN ALBUMIN PREPARATIONS

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In the course of a comparative study of normal and pathological albumins (Charlwood, 1952*a, b*), sedimentation and diffusion studies were made on albumins isolated by electrophoresis from normal human sera. The ultracentrifuge results were lower than might have been anticipated, judging from the publications of previous workers. After examination of the factors involved, it was concluded that the discrepancy lay not in the material, but in an error in determinations of temperature in the earlier work. This conclusion is in agreement with recent measurements on other proteins (Cecil & Ogston, 1948; Kegeles & Gutter, 1951; Miller & Golder, 1952; Taylor, 1952).

The diffusion constants, however, having a mean value of 6.32 units at about 0.2% albumin concentration, were higher than most of the few published figures. Other workers usually gave little detail of their experimental methods, and sometimes omitted to specify concentrations. It seemed that some of the differences might be due to the different methods of isolation of the albumins. To investigate this question, a study has now been made of albumins prepared by three different processes, and the effects of variations in buffer composition have been measured simultaneously.

Slight differences of diffusion constant existed among the normal albumins previously examined (Charlwood, 1952*a*). It is known that, under

various conditions, electrophoresis causes a splitting of the albumin peak (Luetscher, 1939; Hoch & Morris, 1945). This occurs during long separations in veronal buffer. It is clear from Fig. 1, which shows the position of an albumin boundary in the cell immediately before sliding the section, that the relative proportions of the two major albumin components will not only differ from the proportions originally present (quite apart from concentration adjustments caused by the fulfilment of electrochemical conditions, as described by Hoch (1948)), but will depend on the position of the boundary relative to the cell end-plate. This position is determined by the conditions and length of run. The relative proportion of faster component is increased by this effect, which could only be overcome by

Fig. 1. Electrophoretic pattern of albumin boundary given by human serum. Ascending (anode) limb. Migration is from left to right. Veronal buffer, pH 8.6, $I=0.1$. Exposure after 22 hr. 20 min. at 4.7 v/cm.

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