fructose-transferring enzyme system of the artichoke tuber remains an open one. However, the existence of a transfructosidation reaction lends further support to the view that the structures of all the components of the tuber carbohydrates are based upon that of sucrose (Bacon & Edelman, 1951; cf. also Bell, 1947).

SUMMARY

1. From artichoke tubers enzyme preparations have been obtained which catalyse the transference of fructose residues from inulin and associated polysaccharides to sucrose, thereby forming a trisaccharide and higher oligosaccharides.

2. Evidence has also been obtained for the transference of fructose residues to raffinose, melezitose, and to free fructose, but not to maltose, lactose, trehalose or glucose.

3. The liberation of free hexose which took place

in all the preparations examined does not appear to be connected quantitatively with the group-transfer reaction.

4. The reaction, for which the name 'trans-fructosidation' is proposed, is discussed in the light of existing knowledge of hydrolytic and group-transferring enzyme systems.

Note added 22 June 1951. Since this paper was submitted our attention has been drawn to the work of Dedonder (1950, 1951), from which he drew independently conclusions similar to our own about the nature of the artichoke fructosans, and suggested that phosphate was necessary for their synthesis from sucrose.

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The Construction of a Counter-current Apparatus

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The introduction of an apparatus for counter-current extraction by Craig (1944) was a notable contribution to the separation of a wide variety of compounds. Recently Craig (1950) has devised a glass apparatus of large capacity.

In applying the counter-current principle to the separation of lipids it has been found necessary to design a new apparatus in which the fluid phases could easily be examined for emulsions and removed for centrifuging, if necessary. It was desirable that the instrument be suitable for both large and small volumes of fluid. These characteristics have been

embodied in an apparatus of glass and plastic with 49 'theoretical plates'. Its simplicity, and low cost, would seem to adapt it to general laboratory use.

General description of apparatus

The apparatus (Figs. 1, 2) consists of an array of 50 pairs of vertical wide glass tubes with conical ends supported by 'Terry' clips on either side of a wooden frame. The lower ends of each pair are joined by narrow polythene tubing, and neighbouring pairs are joined at the upper ends by wide polythene tubing, the whole forming a continuous spiral around

the frame. Every second glass tube (i.e. all the glass tubes along one side of the frame) has a short open side arm. A bolt at each end of the frame permits the array to be rotated about a horizontal axis.

Each pair of tubes (Fig. 2) corresponds to a 'theoretical plate'. The tubes without side arms (Fig. 2, *A*) are referred to as mixing tubes, and those with a side arm (Fig. 2, *B*) as transfer tubes. To

Details of construction

The general plan of the apparatus is given in Fig. 1, and details of one pair of tubes in Fig. 2. The pairs of tubes are spaced at intervals of 3.5 cm. between centres. The dimensions of the apparatus are adapted to the use of phases of 5–40 ml., of either equal or unequal volume. With the larger

Fig. 1. Schematic diagram of 49 'plate' counter-current apparatus. The tubes are here spread out to show the spiral connexions. *A*, mixing tubes; *B*, transfer tubes; *C*, narrow polythene tubing; *D*, wide polythene tubing; *E*, short open side arm; *F*, short polythene tube and glass rod; *G*, mixing tube with side arm; *H*, metal angle on handle of central frame; *I*, hole; *J*, dropping pin; *K*, butterfly nut; *L*, bolt permitting rotation; *M*, handle of central frame; *N*, wooden frame; *P*, Terry clips; *Q*, joints between tubes and polythene connexions (for details, see Fig. 2).

operate the apparatus the two phases are equilibrated in the mixing tube by rocking the central frame about the horizontal position. Separation into two phases is carried out in the mixing tube, and when the tubes are placed in the vertical position the heavy phase flows into the transfer tube. Rotation of the transfer tube over the mixing tube moves the heavy layer on to the next mixing tube. This cycle is repeated, once for each of 50 pairs of tubes, which are numbered 0 to 49.

volumes the first pair of tubes cannot be used, as tube 0 *A* has a side arm.

Several aspects of the construction merit particular mention. Glass tubing must be selected so that it makes a good fit with the polythene tubing available commercially. For the wide plastic tube (Fig. 1, *D*) we use 22 cm. of polythene tubing of 6 mm. bore (Telegraph Construction and Maintenance Co. Ltd.). Our glassblowers select the wide glass tubing (external diameter 6.5 mm.) so that it makes a snug

fit with the wide polythene tubing. The penalty for too tight a fit is cracking of the polythene.

The narrow plastic tubing (Fig. 1, *C*), 23 cm. long, is polythene tubing (internal diameter 1.5 mm., external diameter 2.5 mm.; Polythene Cannula,

Fig. 2. Sectional view of one pair of tubes. *A*, mixing tube; *B*, transfer tube; *C*, narrow polythene tubing; *D*, wide polythene tubing; *E*, short open side arm; *F*, outer polythene sleeve of joint; *G*, inner polythene sleeve of joint; *H*, narrow glass tube; *P*, Terry clips.

ment over glass tubing. In order that the glass can be sufficiently large to be strong, the narrow polythene tubing is attached to glass of 2.5 mm. internal and 3.5 mm. external diameters, the joint being made by two polythene sleeves. The outer (Fig. 2, *F*) has an internal diameter of 3.5 mm. (No. 55, Polythene Tubing from Portland Plastic Ltd.). The inner (Fig. 2, *G*) has an external diameter of 3.7 mm. and an internal diameter of 2.5 mm. (Telegraph Construction and Maintenance Co. Ltd.). The narrow glass tube (Fig. 2, *H*) may be drawn off the wide tube, or sealed on, though this appears to produce a weaker attachment. Should this latter procedure be used, it is important to select glass by trial with the polythene tubing available, rather than to rely on measurements alone. The polythene-glass joints are sturdy and solvent proof.

The taper of the ends of the glass tubes is satisfactory when made according to the drawing (Fig. 2). More acute taper leads to trapping of fluid, when the tubes are turned.

While tube pairs 1 to 48 are of uniform construction, 0 and 49 differ slightly because they represent the ends of the spiral. In the first pair *A* is not connected to any other tube by its wide end, which is closed by a short polythene tube and glass rod (Fig. 1, *F*). To allow for rapid introduction of solvent, *A* is constructed similarly to *B*, with a side arm (Fig. 1, *G*) which is extended by polythene, a distance of about 8 cm., to the right-hand surface of tube *B*.

The end of the spiral, tube *B* of pair 49, is not connected by its wide end. This ends in a stopcock of 3 mm. bore, the barrel of which is secured by a small rubber ring and peg. The heavy layer of pair 49 is removed through this tap.

Manipulation

To begin a distribution with the apparatus, the required amounts of the two phases are equilibrated

Fig. 3. Diagrams showing positions of tubes during manipulation. *a*, '9 o'clock' (tubes are shaken); *b*, '10 o'clock' (allowed to settle); *c*, '12 o'clock' (height of *B* adjusted); *d*, '8 o'clock' (transfer to next tube).

size 3, from Allen and Hanbury Ltd.). This tubing has been selected because its volume is small, but it is not so narrow that flow is greatly retarded. The dimensions of the narrow tubing preclude attach-

and separated. The manipulation of the apparatus will be described as if the observer were standing at the end of the apparatus, with tube *B* on the right, and *A* at the left.

The apparatus is rotated so that the wide plastic tube is at the 8 o'clock position (Fig. 3d). At this point a metal angle (Fig. 1, *H*) on the handle of the central frame prevents further rotation by impinging on the upright. The two phases, containing the substance to be studied, are run into tube *A* of pair 0 via the side arm of *A* (Fig. 1, *G*). We find it convenient to deliver the phases from a 20 ml. glass syringe, to which is attached a short length of narrow polythene tubing, which passes through the side arm.

To equilibrate the two phases, the central wooden shaft is rotated back and forth about 25° on either side of the horizontal plane (Fig. 3a) so that the two phases flow from one end of the mixing tube *A* to the other. In practice it is found best to dip the narrow end of the tubes rather more than the wide, since otherwise liquid may be trapped in the wide tube. This rocking procedure is carried out for 30–60 sec., at a rate of about 70/min. After mixing, the central frame is clamped by the butterfly nut (Fig. 1, *K*) with the wide plastic tubing in a 10 o'clock position (Fig. 3b).

When the phases have separated the central shaft is turned to the 12 o'clock position (Fig. 3c), and secured there by dropping a pin (Fig. 1, *J*) through *H* into a hole *I*. The heavy layer then moves into transfer tube *B* through the narrow polythene tubing. When this movement is almost complete, transfer tube *B* is raised a little in the Terry clips (Fig. 1, *P*) so that movement stops with the meniscus in the plastic tubing, close to the lowest point, and on side *A*. Once this level has been set, it should not require further adjustment unless the relative volumes of the two phases alter considerably.

In order to move the heavy layer on by one tube, the central shaft is rotated counter-clockwise, to the 8 o'clock position (Fig. 3d). This rotation is made smoothly but quickly, in order that the heavy layer should not pass back through the narrow plastic tube into mixing tube *A*. The cycle is completed in 3–4 min.

Further cycles are now made in a similar way, but with this difference that the new light phase is run into the mixing tube with the advancing heavy layer. This is done via the side arm of the previous transfer tube, since in the present position (Fig. 3d) this leads down through the wide plastic tube to the next mixing tube. Following mixing and separation, when the heavy layer moves from *A* to *B*, only the levels of the two leading transfer tubes have to be adjusted.

A distribution of 49 plates requires approximately 3 hr. In the usual standard procedure the apparatus is turned into the 12 o'clock position and the contents of each pair of tubes are now removed by suction, through narrow polythene tubing introduced via the side arm.

To carry out procedure 1 of Craig (1944), once the

tubes are full the procedure is continued, with the variation that in each cycle one new heavy layer is introduced into mixing tube 0, and one removed from transfer tube 49. This is continued until a desired constituent begins to appear in the heavy layer removed.

In procedure 2 of Craig, the number of cycles is increased until most of the desired material has passed out with the heavy layer. With the apparatus described above, it is possible to run as many plates as is desired. For instance, to run 99 plates on an apparatus with 50 pairs of tubes the standard procedure is followed as above until the apparatus is full. The process is continued, as in Craig's procedure 1, until 50 additional heavy layers have passed through. As these layers come out they are numbered (50–99) and kept separate. The apparatus is then emptied and the first 50 fractions are ready for analysis. The second 50 plates are now run, as in the standard procedure, using new upper layers and introducing the heavy layers into mixing tube 0 in the order which they came out of the apparatus. It will of course be appreciated that bottom layer 50 will become 99 at the conclusion of the distribution and vice versa. The run is complete when tube 49 (now 99) has been reached again.

Fig. 4. Comparison of experimentally determined distribution (O—O) of benzoic acid ($K=2.22$) in a run of 44 'plates', with theoretical distribution (x---x) for $K=2.22$.

One troublesome feature with some solutes is a tendency to emulsification, or in the case of some lipids, even gel formation. Should it be necessary to remove the phases from one tube for spinning, this is readily done in the vertical position by sucking out both phases through the side arm. The apparatus may even be run with three or four tubes removed for

shaking by hand and centrifuging. In this case the appropriate layers have to be withdrawn and returned to the apparatus at each stage.

The apparatus may be used for two 24 plate distributions at the same time, one beginning at tube 0 and one at tube 26.

Effect of solvents on plastic. Although polythene plastics are not entirely resistant to organic solvents, their limitations for our purposes seem few. Mixtures containing light petroleum, benzene, ethyl ether, chloroform, carbon tetrachloride, methylene chloride, methanol and other alcohols have been used.

Faint longitudinal streaks in the narrow plastic tubing and cracks at the joints are occasionally seen, but the tubing is readily replaced.

Performance

As a test of the performance of the apparatus we have employed benzoic acid, in a similar way to Craig (1944). As solvent we have used a mixture of 62 ml. of carbon tetrachloride, 35 ml. of methanol and 3.15 ml. of water. After equilibration the heavier phase is approximately 41% (v/v) of the whole. The distribution constant (K) of benzoic acid in this mixture has been found by titration to be

2.22, at a concentration of 0.5% (w/v), the two phases being of equal volume. Using phases of 20 ml., and 0.196 g. of benzoic acid, a distribution of 44 plates was run, and the amount of benzoic acid in each pair of tubes determined by titration with 0.1 N-sodium hydroxide, using phenolphthalein as an indicator. For comparison, the theoretical distribution of a solute of $K = 2.22$ has been calculated by formulae (3) and (4) from Williamson & Craig (1947). The experimental and theoretical distributions, which are presented in Fig. 4, are in close agreement.

SUMMARY

1. A 49 plate counter-current apparatus of simple construction in glass and plastic is described.
2. Using benzoic acid as a test substance, the performance of the apparatus approximates closely to a theoretical distribution.

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Turacin and the Supposed Catalytic Activity of Copper-Porphyrins

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The role of free copper in the catalytic oxidation of ascorbic acid in foods was first demonstrated by Hess & Ungar (1921-2) and was later confirmed by Euler, Myrbäck & Larsson (1933). The mechanism of this oxidation was investigated by Barron, Meio & Klemperer (1936), who found that ascorbic acid is not autoxidizable in acid and neutral solutions, but that under these conditions it is rapidly oxidized by molecular oxygen in the presence of copper. This reaction is specific for copper and may be used for detecting concentrations of the metal as low as 46 $\mu\text{g./l.}$ The catalytic oxidation of cysteine in pyrophosphate buffer was used by Warburg (1927) for detecting and estimating copper in blood serum, and this method was found to be both extremely sensitive and specific for copper.

In the course of their investigations on the oxidation of ascorbic acid, Stotz, Harrer & King (1937)

found that the reaction took place in the presence of copper-haematoporphyrin and that it was inhibited by sodium diethyldithiocarbamate. Haemin (iron-protoporphyrin) failed to catalyse the oxidation of ascorbic acid. Salomon (1939) claimed that copper-protoporphyrin could catalyse the oxidation of cysteine, its activity on a copper basis being 18.3% of that of copper sulphate at the optimum pH (7.8). On the basis of these results and of those of Stotz *et al.* (1937), it was suggested by Salomon that catalysis by copper-porphyrin might occur without change in valency of the copper.

The main object of this paper is the re-investigation of this problem using turacin, which is the only known naturally occurring copper-porphyrin. Turacin, which constitutes the red pigment of the wing feathers of certain species of the African Turaco bird, was first described by Church (1869, 1892). It was