THE EFFECT OF TEMPERATURE ON CARBOHYDRATE TRANSLOCATION IN THE TOMATO PLANT

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ABSTRACT

The majority of experiments dealing with carbohydrate translocation in the higher plant have indicated a Q_{10} of more than one, that is, a greater transport at higher temperature. Certain of the experiments reported in this work have suggested that under certain conditions, in the tomato plant, a mechanism of translocation may be operative which acts almost independently of temperature, or even shows a Q_{10} of less than one.

In addition to different Q_{10}'s for carbohydrate translocation, vastly different rates of movement have been found, ranging from less than 15 minutes to almost 48 hours, for transport to take place from one leaf to the roots, the growing point, or to the leaves. These experiments have been accomplished by utilizing the valuable tools of radioactive tracers, and a bleeding technique, which is described in the text.

Judging from the variety of results, concerning both temperature effect and rate, it appears unlikely that one mechanism of transport is sufficiently versatile to account for all data. On this basis, more precisely described in the text, the possibility of two mechanisms being operative in the same plant is suggested.

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INTRODUCTION

Translocation of food materials is of importance from the time the seed first begins to germinate throughout the entire life of the plant. Carbohydrates, which are manufactured only in the green cells of the leaf, are needed by every living cell of the plant for the manufacture of other foods, for respiration, for osmotic effects, and for other purposes. Sugar must, therefore, be moved from the leaves to every living cell in the plant.

The problem of translocation is probably one of the most controversial in plant physiology. Despite the vast amount of research accomplished during the two decades preceding World War II, the problem is still essentially unsolved. There still remains a divergence of opinion as to the exact mechanism involved in solute movement.

Little will be said here regarding the general field of translocation. The reader is referred to the monograph of Curtis (1935) and to the reviews of Mason and Phillis (1937) and Crafts (1939) for excellent discussions of the general problems involved.

Probably the first careful observation on translocation was made by Malpighi about 1670, in which he noted that a crude sap ascended from the roots through the fibrous portion of the wood to the leaves. There it became elaborated into a "formative" sap, which spread through the phloem and probably into the bast bundles, to places of growth or storage.

He was led to this conclusion by observing that the seed leaves of a vegetable-marrow plant developed into green leaves not very different from the ordinary ones, and that if they were cut off, the young stem did not grow. Thus he supposed that leaves in general were organs for changing the crude sap brought up from the roots into substances fit for promoting growth.

The fact that carbohydrate translocation occurs primarily in the sieve tubes is well established. Hartig (1837) first noted the existence of sieve tubes: "Long chains of cells whose contents communicate by means of pores in the transverse walls". He later observed exudation from cut phloem and assumed that there was a mass flow of sieve tube contents, as has been more recently postulated by Münch (1930). The work of Schumacher (1933, 1937, 1941) and others has established beyond question that the sieve tube is the principal path for carbohydrate transport.

There is also little question but that sucrose is the chief translocatory carbohydrate in most plants. Went (1944) has shown this to be true in the tomato. The fact that sucrose was found to be the only sugar to show marked diurnal fluctuation in the leaf blade of the cotton plant led Phillis and Mason (1933) to conclude that is was the primary form in which carbohydrates were translocated. Ringing experiments also caused a far more rapid accumulation of sucrose in the leaves than any other sugar, and darkening an isolated leaf resulted in rapid loss of sucrose by the blade, but a gain by

the petiole. Engard (1939) demonstrated that even though sucrose was in considerably lower concentration in the entire raspberry plant (by 10X) than other acid-hydrolyzable and reducing sugars, it nevertheless made the largest accumulations above stem girdles, thus indicating its important role in translocation. Huber (1937) has shown that the primary solid in the phloem exudate of certain plants is sucrose. Smith (1943, 1944) demonstrated that sucrose content changed very rapidly in leaves during active photosynthesis. He found that the carbon assimilated in photosynthesis could be quantitatively accounted for in the carbohydrates formed, between periods of 0.5 and 2.5 hours. At the shorter period, sucrose made up 55.3 per cent of the carbon absorbed. Starch was somewhat less, and monosaccharides accounted for 4.4 per cent. He also noted that sucrose content of the leaves of sunflower plants grown at 10^{o} C. to be about 40 per cent higher than those of plants grown at 20^{o} C., whereas the reverse relation of concentration to temperature held for the hexoses. That sucrose is an early product of photosynthesis, but that there are still earlier products, has been shown very elegantly by Benson, Calvin, et al. (1949). By means of allowing the algae Chlorella and Scenedesmus to photosynthesize for very short periods in the presence of CO_2 containing isotopic C^{14}, these investigators have determined the very first products into which the C^{14} becomes incorporated. After only 5 seconds of photosynthesis, about 70 per cent of the activity is

found in the 3-carbon compound, phosphoglyceric acid. Small amounts of the tracer are also present in triose phosphate, glucose-1-phosphate, and glucose- and fructose-6-phosphate. After 30 seconds, activity becomes established in alanine, malic acid, and aspartic acid, and at the end of only 90 seconds it may be found in the sucrose molecule. The mechanism of these transformations will not be discussed here, but this informative work clearly indicates sucrose as an early product of photosynthesis. By partially submerging the leaves of red clover and wheat in a 10 per cent glucose or fructose solution for 24 hours in the dark, after the plants had previously been kept in the dark for 24 hours, Virtanen and Nordlund (1934) were able to demonstrate the synthesis of large amounts of sucrose.

There is also a certain amount of evidence that sugars other than sucrose are important in translocation. For example, Ripperton (1927) noted that sucrose was the chief sugar in leaves of the canna plant of Hawaii, but that hexoses were present greatly in excess in the stems. Hexoses were considered to be the primary form of translocation. Bulgakova, et al. (1930) found monosaccharides to predominate in the portion of sugar beet plants above ground, but the concentration of sucrose increased steadily from the leaf parenchyma to the roots. The close correlation of length of photoperiod to monosaccharide concentration indicated this form as one of the first products of photosynthesis, and the form in which translocation to the roots occurred.

Environment may exert a profound influence on the type of sugars most common in translocatory tissues. These tissues in the sunflower plant have been demonstrated by Leonard (1936) to have a hexose/sucrose ratio highly dependent on moisture content. The reason for this is not completely clear, although it is probable that the activity of invertase varies considerably with changes in moisture, temperature, pH, etc. The fact that sucrose was always less concentrated in the leaves than in the bark, and that the reverse was true for the simple sugars, led the author to conclude that the latter were converted to sucrose between the leaves and bark.

Although there has been a considerable amount of work accomplished on the general problem of translocation, relatively little has been done on the effect of temperature. Most of the research involving temperature effect on the translocation of carbohydrates has indicated a Q_{10} of more than unity at temperatures below 30° C., that is, an enhanced translocation at higher temperatures. Some of this work will be briefly cited.

Using red kidney bean, Curtis (1929) found that cooling the petiole to 1-6° C. greatly retarded translocation from the leaf during darkness, as determined by loss of solids. When the petioles were chilled only to 9-10° C., however, transport was approximately the same as in controls at 20-25° C. In a later paper (Curtis and Herty, 1936) it was found that this loss of dry weight from leaves of chilled

petioles was highly dependent upon the time of chilling. A 17 hour period of chilling gave a considerably greater amount of transport at the lower temperature than did a 9 hour period, but even with a 4½ hour exposure at the low temperature a significant amount of transport still took place. The above relations held regardless of whether just the petioles were cooled or the entire plants were placed at the lower temperature. In one of the experiments, each of which involved temperatures in the high, medium and low ranges, the translocation at all three temperatures was almost identical, and was significantly greater than losses from the control leaves which had scalded petioles.

Using an improved technique and a greater range of temperatures, Hewitt and Curtis (1948) investigated the loss of dry matter and carbohydrates from leaves of bean, milkweed, and tomato. Respirational losses during a thirteen hour dark period at temperatures ranging from 4° C. to 40° C. were determined by analyzing paired leaves -- one cut at the beginning of the period, and the other cut at the beginning but analyzed at the end of the period, the difference in carbohydrate content being attributed to respiration. Translocatory losses during the same period were determined by also leaving an intact plant under the same temperature conditions, removing one leaf at the beginning of the dark period for immediate analysis, and the paired one at the end of the period. Difference in carbohydrate content was attributed to translocation, which was found to be greatest

at 20° C., and fell off considerably at higher and lower temperatures. By separating leaves, stems, and roots both before and at the end of the dark period, and analyzing all at the end of the period, the differences can be ascribed to translocation. It was found that the roots gained most at 20° C., whereas the stems lost at all temperatures, but slightly more at 20° C. The leaves lost almost equally at 20°, 30°, and 40° C. and somewhat less at 10° C. The authors consider that the comparatively small translocation losses at 30°, and especially at 40° C. may be due to the smaller amount of carbohydrate available for transport, since it is largely used up in respiration at the higher temperature.

Utilizing the bean plant, Swanson and Böhning (1951) have recently obtained some interesting results regarding temperature effect on the rate of carbohydrate transport, in the range of 5° C. to 40° C. Transport was measured in terms of rate of elongation of the stem and first trifoliate leaf. By the use of special temperature jackets, only the temperature of the petiole was varied, the other parts of the plants being maintained at 20° ± 1° C. for all treatments. During the experimental period of 4 to 5 days, the plants were kept in total darkness, sucrose being supplied to the plants by immersion of one leaf blade of the temperature treated leaf (the only one left on the plant) in 0.75 molar sucrose solution. The maximum transport, as measured by elongation of stem and leaf, occurred at petiole temperatures

in the range of 20° - 30° C. At temperatures of 5-7.5° C., the rate of transport was diminished 20-45 per cent, this being significantly lower than the optimum rate only at the 5 - 6 per cent level. The rate at the higher temperatures of 40° - 42° C. was diminished 25-75 per cent, which was very significantly lower than the optimum rate at 20° - 30° C. The rate-temperature curve superimposed favorably with that established by Hewitt and Curtis, which has been described, but the latter authors' findings indicated a considerably greater depressing effect of low temperatures than did those of Swanson and Böhning. It was interesting that at a 20° C. petiole temperature elongation rate remained almost constant throughout the five days, but at lower temperatures, the retarding effect on translocation progressively decreased with time, whereas at higher temperatures it progressively increased. This apparent acclimation at the lower temperatures with increasing time is not unlike the phenomenon discovered by Child and Bellamy (1919), to be presently discribed.

Crafts (1932) found that chilling the stems of Cucurbita to 2° - 4° C. for several days caused about a 50 per cent reduction of phloem exudation from the cut stem. When the entire plant was chilled, even a greater retardation of exudation was found. This was explained by the author as being due to an increased viscosity of the solution of nutrients, the retardation therefore being proportional to the length of conducting channel chilled. One should, however,

not confuse exudation from cut or injured stems with true translocation, even though he considers translocation to occur by a similar type of mass flow. The total composition of the exudate was not significantly different in the chilled and unchilled plants. The fact that protoplasmic streaming is completely stopped by temperatures used in this experiment would seem to indicate that protoplasmic streaming as a mechanism for solute movement, as first suggested by deVries (1885), is completely untenable, at least as a primary mechanism. Even at higher temperatures where streaming does occur, its rate is far too slow to account for reported rates of solute movement. Also it is very questionable that streaming takes place in the mature sieve tube at any temperature.

Using the non-toxic dye fluorescein, Schumacher (1933) observed its movement through Pelargonium and other plants by means of ultraviolet light. After application in a gelatin block to either a leaf or stem surface which had the cuticle removed, the dye could be followed through the protoplasm of the sieve tubes. Movement in this manner was found to be at a rate of 20-30 cm. per hour at temperatures of 20° - 30° C. to which the entire plants were exposed. At 1° - 4° C. the movement was reduced to only 1-3 cm. per hour. When first applied on the leaf surface, at 30° C. the dye passed through the entire petiole in one hour. At 11° - 12° C. it took 3-4 hours for detectable amounts to pass even into the veins, and at 1° - 4° C. 10 hours were required before

movement into the vein could be detected. After this long period, movement suddenly commenced and the dye was carried throughout the plant at the rate of 1-3 cm. per hour mentioned above. The author considers that the data do not support a protoplasmic streaming mechanism since streaming is much slower than the rates observed, but suggests that a living protoplast within the sieve tubes is a requirement for the translocation of fluorescein. It should be kept in mind that fluorescein is a foreign substance to the plant and therefore its transport may not follow the laws of certain other naturally occurring products.

By chilling the petiole of Biloxi soybean to 3° C. during a four day photoperiodic induction period, Parker and Borthwick (1941) noticed that export of the flowering hormone from this one leaf (all others having been removed from the plant) was greatly inhibited, as determined by the number of flower primordia later forming on the plant. Also, the increase in length of the center leaflet of the lowermost leaf in the terminal bud was about 2-8X greater in control plants compared to those with the one petiole chilled to 3° C. Apparently the cold treatment reduced the translocation of carbohydrates traveling from the one leaf to the growing point. When the growing point was cooled rather than a leaf petiole, the same general inhibition of floral primordia appearance held. Increase in length of the young leaflet mentioned above, within the cooled tip, was even considerably less than when the petiole of a mature leaf was

cooled instead, clearly indicating the strong inhibitory influence of cold on the growth process itself. In all of these experiments, the temperature required to reduce materially the amount of floral initiation was about 3° C., very little inhibitory effect being found at the range of 6° - 10° C.

In a more recent experiment, Parker and Borthwick (1943) demonstrated that when only one leaf blade (rather than a petiole) was chilled during the five day induction period, while the rest of the plant was on long days and at normal temperature, only 7° C. served to almost completely inhibit formation of floral buds. There was also an inhibition of flower bud formation when the leaf was exposed to temperatures above 32° C. for five days during its short day induction period. The greater sensitivity of the leaf blade to cold temperatures than the petiole, may well indicate that the cold effect is primarily on the photoperiodic reactions occurring in the leaf blade, rather than on translocation itself.

Buds in the cotyledonary axils of the scarlet runner bean and the Lima bean normally never develop. However, Child and Bellamy (1919) have found that if a 3 cm. section of stem above the cotyledons and below the first pair of leaves is cooled to 3° - 5° C., the buds do develop, and the plant above the cooled zone appears normal or at most is only slightly retarded in growth for two or three days. Release of inhibition on buds farther up the stem may be likewise accomplished by cooling the stem immediately above.

It is interesting that 6° C. was found to be about the upper limit of effectiveness -- about the same threshold value as certain other temperature phenomena in the plant. After several days the buds would again become inhibited due to an apparent acclimation of the cooled zone, which occurred more readily at 6° C. than at 3° C.

An interesting corollary was later noticed by the same authors (Child and Bellamy, 1920) with Bryophyllum. When the leaves were partially submerged in water or placed in moist air and cooled about the petioles to 2.5° - 4° C. for a few days, there later arose a great increase in the outgrowth of plantlets about the periphery of the leaves. Submergence of the leaves alone caused only a very slight increase in outgrowth. Cutting of the petiole or compressing it to one-half of its original diameter with a screw clamp also augmented the outgrowth, but to a much lesser extent than chilling of the petioles. The leaf opposite to the one treated usually had almost as great an outgrowth as the treated leaf, and leaves at adjacent nodes above and below the treated one also developed extra plantlets, but to a lesser degree than the treated leaf. The significance of this experiment, and the question as to whether the inhibitory influence of chilling was on the carbohydrates or on an auxin will be left to the discussion.

In addition to the experiments previously described, which essentially indicate a Q_{10} of more than one for solute and carbohydrate transport, there is considerable evidence for a Q_{10} of unity or less. Some of these

experiments will now be briefly reviewed.

Evidence for a Q_{10} of one, for the velocity of transport of auxin in the Avena coleoptile, has been demonstrated by van der Weij (1932). By measuring the transport through a coleoptile section interposed between a donor and a receptor agar block, he found that amount transported per unit of time increased rapidly from 0° C. to 35-40° C. and had a Q_{10} close to 3, whereas the velocity was completely independent of temperature.

The data of Kruseman (1931) also tend to indicate a Q_{10} of about one for carbohydrate translocation in the case of Phaseolus multiflorus Lmk. Using simply the iodine test as an indicator of starch, his early experiments showed an inhibited translocation when the petioles were chilled. Later work, in which translocation was determined as a function of change in dry weight per unit of leaf area, gave variable results and failed to show any correlation with temperature when the petioles were cooled to temperatures between 3° and 11° C. Kruseman did conclude however, that the living protoplast exerted some influence on the process of translocation.

Montemartini (1928), in working with Ceratonia siliqua, found photosynthesis to be very weak at 3° C., but that translocation was not stopped until the temperature was below 0° C.

By utilizing early flowering Chrysanthemum as a long-day plant (actually the early flowering variety is indeterminate) and the late flowering variety as a short-day plant,

Grainger (1938) noticed that the late flowering variety contained a larger proportion of starch. In such plants, this starch was not hydrolyzed to reducing sugar at all during the short nights of early and mid summer, and only by artificially increasing the length of the night during mid-summer was the late flowering variety found to contain some reducing sugars toward the end of the night (which were soon lost by translocation and respiration), and made to flower early. The long-day plants, on the other hand, have reducing sugar present during most of their 24 hour cycle, much of which is lost during even the relatively short nights of early summer. This would seem to indicate that translocation takes place essentially at night. It of course is open to question whether this greater nocturnal translocation is primarily a result of lower night temperatures or of an increased accumulation of photosynthetic assimilates toward the end of the day.

Tschesnokov and Bazyrina (1930) determined photosynthesis of pea and potato plants by CO_2 uptake, as well as making studies of respiration and translocation by noting changes in dry weight. The course of translocation during day and night was found to be very different in the two plants. Translocation in the pea was simultaneous with the maximum intensity of photosynthesis in the early afternoon, and decreased toward evening, although continuing at a slow and uneven rate throughout the night. In certain cases it ceased completely at night. The potato, on the

other hand, was found to exhibit translocation primarily at night, beginning at 16:00-18:00, reaching a maximum about 20:00, and continuing all night. It was absent during intense photosynthesis of the daylight hours. Since the potato was found to accumulate insoluble carbohydrates in the leaf, whereas the pea accumulated soluble sugars, it seems very probable that there is a relation between the type of storage material present and the course of translocation. The author also noticed that monosaccharides did not change a great deal during the day and night, as did sucrose, these findings being confirmed by Went (1944b) in the case of tomato. The reason for these differences between day and night translocation is rather obscure. Although the type of storage material is undoubtedly important, a temperature function would also appear quite likely, the former of course being somewhat dependent on the latter. Further investigation must be carried out before the answer can be found.

Upon growing various plants at controlled temperatures, Arthur, et al. (1930) found that lower temperatures of about 20° C., compared with temperatures of 26° C., caused a considerably enhanced tuber production in the Irish Cobbler Potato. The higher temperature produced heavier aerial portions but little or no tuberization. An increased photoperiod and light intensity combined with high temperature increased the growth rate only of the aerial portion of the plant, whereas if combined with a lower temperature, it

primarily increased tuber production. Lacking further data, it is difficult to say whether the deficient tuberization at high temperatures is due primarily to an inhibition of translocation at such temperatures, or primarily to increased respiration and utilization of carbohydrates within the aerial portion of the plant, thus leaving little or no surplus available to the roots. A careful respiratory study of this plant under different environmental conditions is certainly indicated in order to determine whether respiration is a major factor here or not.

Some interesting findings regarding translocation of carbohydrates into date fruits have been published by Curtis (1947). He noted that dry weight of the fruits increased considerably during the night due to influx of sugar, whereas little or no increase was found during the day. It was shown that less than 10 percent of the net daily gain in weight was consumed in respiration during the daylight period. It is known that the date palm stores a large amount of starch in the trunk which is hydrolyzed to sugar during the late summer, and furnishes a supply of carbohydrates for the fruit. Thus, with a constant source of carbohydrates, translocation into the fruit would depend upon the efficiency of the transporting channels during the day and night, and accumulation in the fruit. It would not depend upon photosynthesis with resulting diurnal fluctuations in concentration of assimilate, as was probably the essential cause of Mason and Maskell's (1928) finding in the cotton plant of accumulation of dry material

in the cotton boll chiefly during the day. The most likely explanation for this phenomenon would seem to be that the high day temperatures inhibited translocation more than the lower night temperatures.

Goodall (1945, 1946) did a considerable amount of experimentation on tomato plants, working primarily on change in the dry weight of various organs, both intact and separated from the plant, and on translocation rates. He found roots to show an increase in dry weight during the daytime and a loss during the night in summer, but in winter they remained constant during the day and increased during the evening. This would seem to indicate that in summer, movement of material formed during the day is almost completed before nightfall, whereas in winter, translocation is greater during the night. Also, during the winter evenings the young leaves gained at the rate of 2.77 per cent per hour in dry weight, while the plant as a whole decreased in dry weight. During the summer evenings however, the same type of young leaves lost rapidly -- about 3 per cent per hour. This would seem to confirm the impression that in winter translocation continues throughout the night, but ceases at dusk during the summer. As to the stems, there was practically no change in the dry weight between dusk and midnight, the loss by respiration apparently just being balanced by translocation to the stem. Between midnight and dawn however, there was a marked increase in dry weight due to an increased translocation. This relation was found during the summer, but not during the winter. It is

difficult to say whether this enhanced translocation during the pre-dawn hours was due principally to the colder temperatures to which the plants were subjected at that time during the summer, but not during the winter when the greenhouses were kept heated at a more or less constant temperature all night, or whether it was due simply to a different lag period resulting from different initial concentrations of assimilate formed during the summer and winter. At any rate, these experiments give a strong indication of an increased translocation rate at lower temperatures. A statistical analysis of the effects of several environmental factors upon the rate of translocation showed that the rate was inversely proportional to the temperature, indicating a Q_{10} of less than one. This inverse proportionality, although small, was statistically significant when the means of the different experiments were considered.

In addition to the experiments just described, most of which suggest a Q_{10} of one or less in the higher ranges of temperature, there is also evidence that the same Q_{10} relationship holds under certain conditions at temperatures even down to 1° C. Utilizing tomatoes grown under controlled conditions, Went (1945) has shown that although the optimum temperature for the growth process lies around 30° C., the optimum night temperature (when most of the stem elongation occurs) is considerably lower, probably being due to a decreased sugar translocation at higher temperatures. As

the plants became taller, translocation would become more and more limiting, and consequently the optimum night temperature for stem elongation would shift to a lower level, in accordance with the above mentioned statement. The same phenomenon has also been shown in the case of the chili pepper, Capsicum annuum (Borland and Went, 1947). The optimum night temperature was found to decrease as the plant matured, from 30° C. to 8.5° C.

If translocation from leaves to roots were greater at low temperature, one would expect an inhibition of root growth at the higher temperatures. Actually, the roots are considerably lighter, the higher the temperature -- this, despite the fact that a teleological explanation would indicate a large root system for plants grown at a high temperature, and which would transpire accordingly at a high rate. Since tomato plants grown at night temperatures above 18° C. are limited as to sugar translocation, all sugars are immediately used by the growth process as soon as they arrive. If such plants are transferred to a 26° C. night temperature, they assume the growth rate common to such a temperature. If, however, they have been grown at night temperatures below 18° C., and are then transferred to a 26° C. night temperature, there is a surge of growth above what would be common at the 26° C. night temperature, due apparently to utilization of the sugar which had accumulated at the lower night temperature.

When different parts of the tomato plant were analyzed for sucrose at short intervals throughout the night (after

a normal day in the greenhouse), some interesting relationships were demonstrated by Went and Engelsberg (1946), especially when the plants were kept at different temperatures during the night. The young full-grown leaf blades had somewhat less sucrose throughout the night if plants were kept at 17° C. as compared to 26° C. Plants kept at 8° C. however, contained somewhat more sucrose in the blades, at first showing a rapid rise in concentration from 18:00 up to about 24:00 due, apparently, to the conversion of some other metabolite into sucrose at the low temperature, and then followed by a rapid decrease in sucrose content between 24:00 and 8:00. Since one would expect little respiration at this low temperature, the rapid disappearance of sucrose after midnight must be accredited mainly to translocation out of the leaf. The relationship is shown in Table I.

TABLE I

Sucrose Content in Per Cent of Dry Weight of the Leaf Blades of the 3 Youngest Full Grown Leaves.

	Night Temperature, ° C.		
	26°	17°	8°
18:00-24:00 (Mean of 18, 20, 22, 24:00)	0.79	0.90	1.63
04:08-08:00 (Mean of 4, 8:00)	0.67	0.52	1.14
Difference (Translocation)	0.12	0.38	0.49

If respiration were considered, it would be greater at the higher temperatures, and thus if deducted would give an even

greater relative difference in translocation at the different temperatures. This experiment gives almost indisputable evidence for a Q_{10} which is less than unity. It was also found that at a night temperature of 8° C., the sucrose content of the roots dropped somewhat between 16:00 and 19:00 but increased by about 200 per cent between 19:00 and 08:00 the next morning. At the higher temperatures of 17° C. and 26° C. the sucrose content steadily dropped off during the entire night, slightly more at the latter temperature. Even if accumulation of sucrose in the roots at low temperature is partially due to a decreased utilization in both leaves and roots by respiration and growth, the fact that the increase in sucrose content of the roots so closely parallels the rate of loss in the leaves suggests very strongly that the root carbohydrate is derived from the leaves, and that its rate of translocation is greatest at the lower temperatures.

It had previously been demonstrated (Went, 1944b) that in general, when tomato or lettuce plants are kept at a relatively warm temperature during the night, the following morning they invariably show a higher sucrose concentration in the leaves. For example, when kept during the day and night at 26.5° C., the leaves of tomato plants showed at 08:00 a sucrose content of 1.59 per cent of the dry weight, whereas if the night temperature was lowered to 19° C., the value dropped to only 0.96 per cent. This difference is found in spite of the fact that one would expect a higher respiration at the higher night temperature and consequently a lower residual amount of sucrose in the leaves. Since

actually a smaller amount of sucrose is found in the leaves of the plants kept at the lower night temperature, it is a strong indication that a larger amount of the sucrose is translocated out of the leaves during the night at the lower temperature, and consequently indicates a Q_{10} of less than one. Reducing sugars and starch were also analyzed for in these experiments, but showed very little relation with temperature, as did sucrose. It was also shown that if tomato plants were girdled by steaming a short section of the stem, the entire plants then being placed at either 26.5° C. or 18° C. in the dark for 24 hours, a small but significant amount of sucrose would accumulate only above the girdle of the cooler plants. Two different experiments gave essentially the same results, thus indicating an increase in translocation at lower temperature. One of the experiments is shown in Table II.

TABLE II

Sucrose Content in Per Cent of Dry Weight of Tomato Plants kept for 24 Hours in Darkness at 26.5° C. or 18° C.

	18° C.		26.5° C.	
	Girdled	Non-Girdled	Girdled	Non-Girdled
Above Girdle	3.49	3.70	2.65	2.93
Below Girdle	3.10	3.65	2.67	2.90

A similar experiment was also performed, in which the volume of exudate was measured rather than the sucrose concentration. Measurement of exudate makes an excellent tool in the study

of translocation. Its use will be later described. Tomato plants were girdled or not girdled and placed for 24 hours in the dark at either 18° C. or 26.5° C., just as in the last experiment. At the end of this period all plants were decapitated and placed at 26.0° C., during which time the exudate was collected over the 50 hour period following and also a later 42 hour period, and measured. The girdles effectively interfered with the downward translocation of substances necessary for the production of bleeding, and indicated that living cells are essential for this translocation. Data for the exudate collected during the first 50 hours is shown in Table III.

TABLE III

Volume of Exudate in cc. Given off during 50 Hours Following a 24 Hour Pre-treatment in Dark at the Temperature Indicated Below.

	18° C.	26.5° C.
Girdled Plants	4.60 ± 0.48	3.44 ± 0.33
Non-girdled Plants	8.47 ± 0.43	4.62 ± 0.59
Plants decapitated at time of steaming	9.76 ± 0.87	

From this relationship it would appear that the significantly greater amount of exudation occurring in plants kept at the cooler temperatures is due to a greater amount of osmotically or metabolically active material in the roots, this material apparently having been translocated to a greater extent at the lower temperatures. Even though

respiratory losses in the aerial portion of the 26.5° C. plants would be somewhat greater than in the 18° C. plants, the difference would be small, and it appears very unlikely that the volume of exudates would be a function only of the residual carbohydrates. i.e., with a higher respiratory rate, there would be less residual carbohydrate left to be translocated to the roots. The possibility of interconversion of non-osmotically active carbohydrate into osmotically active carbohydrate within the roots at 18° C. can not be ruled out. However, previous knowledge of temperature-carbohydrate relations makes it appear most unlikely that such a conversion would take place at a temperature as high as 18° C., and in such a short time. Another bleeding experiment clearly demonstrated that the amount of exudate coming from decapitated plants having been previously kept at different temperatures, is not dependent upon variable interconversion of the carbohydrates within the roots at the different temperatures, but rather is dependent upon the amount of material previously translocated from the aerial portion to the roots. This was done by decapitating the plants 5 cm. above the ground and placing them for 24 hours in the dark at temperatures ranging from 8° C. to 26.5° C., along with controls which were not decapitated. At the end of this period, the intact plants were decapitated and drip tubes were connected to both sets of plants. The difference in exudate collected over the next 48 hours between the pre and the post-dark period decapitated plants was compared for each of several temperatures. The difference

at any given temperature was taken as a measurement of carbohydrate translocation into the roots which had occurred in the intact plants during the 24 hour dark period. This difference was very great at 8° C., and decreased in a linear fashion to a very low value at 26.5° C.

Thus we see that there exists a rather wide array of experimental results, as far as temperature effect on translocation in concerned. The controversy regarding the exact mechanism involved in organic solute movement, which will later be discussed, is probably even more unsettled. It is hoped that various types of experimentation involving temperature effect on the translocation of carbohydrates may eventually create one more building block which will be useful in the final solution of the translocation problem.

EXPERIMENTAL

Bleeding -- A useful Tool in the Study of Carbohydrate Translocation

Literature Review

Most plants will exude a sap if they are injured or decapitated, this exudation usually being from the transpiration stream moving up the xylem. Tomato plants, providing they are in good condition and well watered, may bleed in this manner for five days or more, after having been decapitated. Went (1944b) first demonstrated that tomato plants, after having been partially depleted of carbohydrates by 24 hours of darkness, and then decapitated, will bleed considerably more during the next two days if one or two of the leaves on the stump are placed in a solution of 5 to 10 per cent sucrose. This clearly indicates that sugar limits exudation in partially starved plants, and that bleeding rate may be expected to depend on the amount of respiratory metabolite present. Thus by carefully noting the rate of bleeding over an extended period, one should have an accurate measure of the extent of carbohydrate translocation from other parts of the plant to the root.

When the tomato plant is kept in the dark for a longer period of 48 hours, Dubnoff (1944) has shown that only a slight increase in exudation takes place when the leaves are submerged in a 5 per cent sucrose solution. The plant apparently reaches a certain degree of starvation beyond which it is unable to utilize an external source of carbohydrate. It will still recover at this point however, if

returned to light.

It was also found that if all leaves of a plant except one were removed before a 24 hour dark period, the plants bled less during this period than if they had not had the leaves removed. The one-leaf plants, whether the leaf was submerged in a sucrose solution or not, bled significantly more if the temperature during the 24 hour dark period was 7° C. rather than 18° C. This phenomenon held true in spite of the fact that absorption of water by the plant under conditions of low soil temperature is considerably decreased, as shown by Kramer (1940) in the case of sunflower and privet, and by the present author in the case of tomato. Grossenbacher (1939) also found that the sunflower bled very little at 10°, more at 15° and considerably at 30°, but slowly dropped off after several days at the higher temperature. The increased bleeding noted by Dubnoff at the lower temperature must be due either to a general conversion of carbohydrates to their most osmotically active form within the stem and especially within the root, or to a greater translocation toward the root at 7° C. than 18° C. The phenomenon can not be ascribed to the loss of assimilates through respiration at the higher temperature, because the increased bleeding rate at the lower temperature is apparent almost from the beginning -- long before any significant amount of assimilate could be lost through respiration at either temperature.

The manner and form in which externally supplied carbohydrates are absorbed and translocated is of interest. Said (1948) has found that when cut ends of barley leaves or slices of storage organs are dipped in sucrose solution, they do not absorb sucrose as such, but only the products of inversion. Evidence will later be presented which indicates that products other than the hexose sugars are also translocated when sucrose is applied to the leaves.

Bleeding in itself is an exceedingly intricate and sensitive process -- a process which is not well understood. If one considers two plants which are of identical genetic background, of identical age, and which have been grown side by side under the same environmental conditions -- plants which to the eye are identical twins, it may nevertheless be that when these plants are decapitated one will bleed profusely whereas the other will not bleed at all. Such is the case in tomatoes, and is the reason why large numbers of plants must be utilized to establish results which are statistically significant. A satisfactory explanation for the variability has not been found.

Bleeding has been variously ascribed to osmotic forces and to active or "vitalistic" forces. Experiments involving temperature and narcotic or anaerobic effects have generally indicated that energy for bleeding is provided by respiration. Working with excised root systems of tomato, vanOverbeek (1942) found root pressure to be made up of two components:

an osmotic factor, and an active factor, the latter being reversibly inhibited by 10^{-4}M KCN. The active factor was described as being dependent upon respiratory processes, in conjunction with heavy metal catalysis. Heyl (1933) utilizing decapitated stems of Sanchezia, Ricinus, and Brassica also demonstrated that exudation of sap from the cut surface could be inhibited by hydrogen or narcotics such as ether and chloroform. These experiments indicate that the bleeding mechanism is located in the roots and involves active excretion of water by living cells. A weak electric current was also found to enhance the bleeding whereas a stronger one hindered the process. Heyl also concluded that the mechanism of bleeding must depend upon certain processes which are not entirely osmotic in nature.

On the other hand, Eaton (1943), by investigation of osmotic values of bathing solution and exudate under different conditions, came to the conclusion that simple osmosis and capillary forces account for root pressure and bleeding of the tomato plant, and that there is scant basis for the view that vital activity is involved. This view, however, is not supported by Skoog.

Actual pressures developed by the root system are very considerable. White (1938) found that tomato roots in culture solution, when attached to a manometer with six atmospheres of pressure applied, were not appreciably retarded in secretion, thus indicating that the sap movement takes place under pressures considerably exceeding this value.

Upon walking over a wooded area which had been partially logged four years previously, Friesner (1940) noted a striking exudation through the uninjured stems of Acer rubrum, one to two inches in diameter. The exudation came only from stump sprouts, none being found from virgin timber. The stumps were 3 to 5 inches in diameter. This observation does not indicate that osmotic activity of living cells in the stem is not involved, but certainly suggests that root pressure is a factor of great significance. It is interesting to note at this point Schumacher's finding that root pressure has no effect upon the velocity of fluorescein transport.

There is a natural periodicity in the bleeding rate of most plants, with a maximum usually about noon and a minimum during the night. A diurnal fluctuation of this type in root activity was probably first reported by Hofmeister (1862). He found that although such fluctuations could occur independently of environmental changes during the time of observation, they could however be modified by such changes. Bleeding experiments carried out by Speidel (1939) on Helianthus, Ricinus and other plants clearly demonstrated the existence of a daily periodicity. Bleeding was found to be dependent on time of cutting and temperature. Also using Helianthus, Grossenbacher (1939) found that the diurnal cycle of exudation was independent of the time of day that the plants were decapitated, provided they had been growing under normal greenhouse conditions. Under constant temperature of 20° C. and continuous light of either very low or 300 foot

candle intensity, the plants exhibited a 24 hour cycle of maximum and minumum exudation, but the first peak was about 12 hours after depacitation, regardless of what time it was made. The following peaks were spaced at 24 hour intervals from the first peak.

White (1938), using tomato plants grown in culture solution, noted that upon decapitation they would continuously and rhythmically secrete sap. Experiments of Went (1944b) in which an accurately measuring bleedometer was used, and later experiments of the author have demonstrated a maximum usually around 11:00, in the case of tomato when kept at a constant temperature during the bleeding.

That bleeding tends to be rather erratic and not well correlated with any other known function has been found by different investigators. For example, Krasovskaya (1947) uses the volume of flow of exudate from the cut stump per unit of time as a measure of root system vigor. She finds absolutely no correlation between root weight and quantity of exudate, but did note that exudate per unit of fresh weight of tops was less in shade plants than in sun plants. Dubnoff (1944) also failed to demonstrate a proportionality between rate of bleeding and root weight, and found but slight correlation between bleeding rate and sucrose content of roots, whether or not the plant had been fed sucrose through the leaves.

In spite of the irregularity of the bleeding process and of its failure to correlate well with any known function

or quantity within the plant, it nevertheless becomes an exceedingly useful tool when properly used. A recent study was made by Went and Hull (1949) on the effect of temperature on carbohydrate translocation, utilizing the bleeding phenomenon as a primary tool. Tomato plants of the San Jose Canner Variety were used for the experiments at an age of about two months, when they averaged around 75 cm. in height. All leaves were pinched off from the lower 22 cm. of the stem to allow installation of cooling collars. This was done at least four days before the experiment, since it has been shown (Went and Carter, 1945) that removal of the leaves during application of sucrose solution to two leaves reduces the bleeding stimulus considerably, as well as subsequent growth. The effect is more marked when the leaves are cut than when they are pinched off, but decreases markedly after several days. The two leaves remaining immediately above the removed leaves were then submerged in 7 per cent sucrose (or in water as a control), and the plant was decapitated just above these two leaves. Rubber tubing which led to a dripping recorder was connected to the decapitated stump in such a way as to make any change in the rate of bleeding immediately visible. It was found that if the leaves were submerged in 7 per cent sucrose there was a sudden increase in the rate of bleeding after about 11 hours at 23° - 24° C., as compared to control plants with leaves in water. If, however, the stem was chilled to 1° C. by means of an insulated metal collar in which cold water was circulated

(through copper tubing soldered to the collar), it was found that only about 8 hours were taken instead of 11 for this increase in rate of bleeding to be noted. The relationship is shown graphically in Fig. 1. Intermediate temperatures between 1° and 24° C. gave lag periods more or less intermediate between the extremes, as indicated in Fig. 2. This inverse relation would seem to indicate that sucrose or at least some metabolically active substance is translocated into the roots, thus causing the increase in rate of bleeding and that the rate of downward translocation of this substance is greater at the lower temperature.

In considering only plants which had either 7 per cent sucrose or water supplied to the leaves, but not those which had cooled stems, reducing sugars[1] of the roots of plants supplied sucrose were equal to or greater by about 10 to 30 per cent than those of the plants supplied only water, in nine cases out of ten. In the case of the sucrose content of roots however, there appeared to be no correlation between control plants and sucrose-fed plants. This strongly suggests that the added sucrose reaches the roots in the form of hexose sugars, but gives no enlightenment as to the locus of inversion. The fact that one mole of sucrose upon inversion yields two moles of hexose, and consequently twice the

1. The determination of reducing sugars in these experiments was not made from extracts cleared of non-sugar reducing substances with lead acetate, and consequently a small amount of the reducing power must be due to these substances. Sucrose was determined simply by the difference in reducing power before and after inversion with invertase, reducing power being determined according to the method of Hassid (1937).

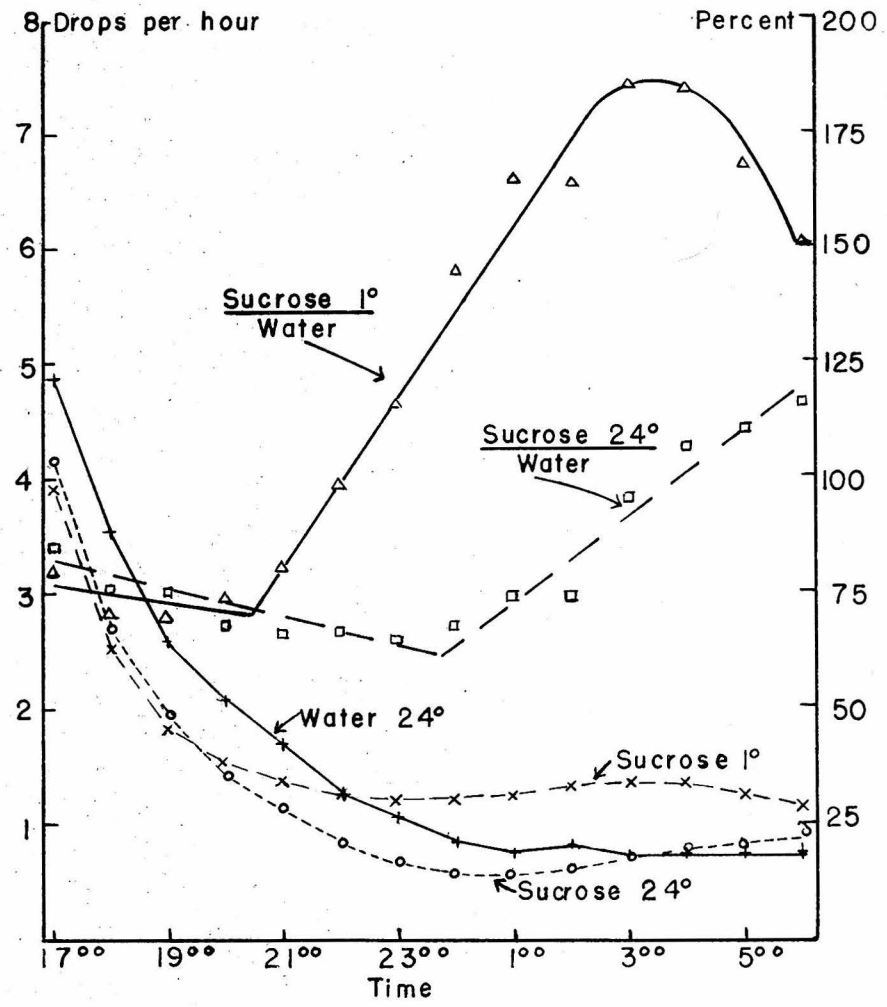


Fig. 1. Rate of bleeding of plants having two leaves in water or two leaves in 7 per cent sucrose. Stems of one group of sugar-treated plants cooled to 1° C. The proportion of the rate of bleeding of both groups of sugar-fed plants in per cent of that of the water controls is plotted. (After Went and Hull, 1949)

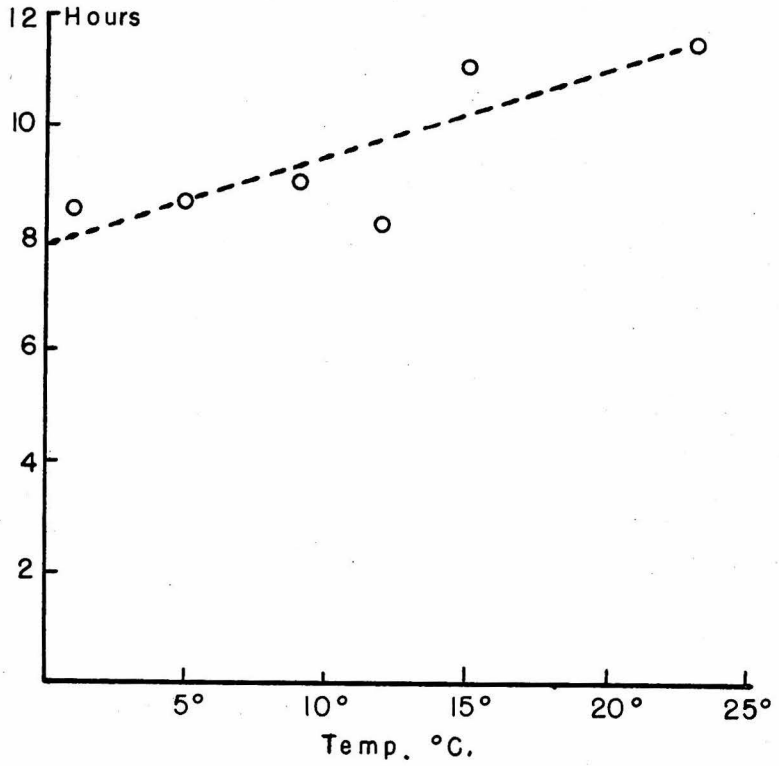


Fig. 2. Relationship between temperature of the stems of the sugar-fed plants and the length of time it takes before the effect of the applied sugar can be observed. (plot includes all experiments) (After Went and Hull, 1949)

osmotic activity should not be forgotten in explaining the greater bleeding rate of the sucrose-fed plants.

If only the plants fed sucrose are considered, which had their stems either cooled or at room temperature, it is seen that in 16 out of 17 experiments a higher sucrose content existed in the roots of plants with stems maintained at room temperature. The average difference in sucrose content of the roots between these two series was 1.6 mg. per g. of dry weight. Reducing sugars of these two groups showed no correlation, being slightly greater in roots of cooled stem plants in about half of the experiments and slightly greater in the roots of the room temperature group in the other half. This relationship within the sugar-fed plants indicates an apparent negative correlation between sucrose content of the roots and bleeding rate. However, it must be considered that the plants were always harvested after the excess bleeding rate caused by sugar application had tapered off to that of the controls. The fact that the analyses were not carried out when the differences in bleeding rate were most pronounced would tend to invalidate the above inverse correlation considerably. In general, all sugar analysis data showed a definite trend toward an inverse correlation between sucrose content and reducing sugar content of the roots.

Experimental Methods

Certain other experiments were carried out by the author in order to determine optimum conditions for bleeding. Tomato plants var. San Jose Canner or Marglobe used in these experiments, unless otherwise stated, were grown in two large concrete tanks containing Hoagland's nutrient solution. The solution was maintained at 28° - 30° by means of electric immersion heaters. By use of such elevated nutrient temperatures, Gericke and Travernetti (1936) were able to grow tomatoes at the rate of one ton a year in a basin area of 100 sq. ft.

A special metal trough was constructed from which twelve plants could be bled, and the rate of bleeding recorded simultaneously. Water in the trough was slowly circulated by means of an electric stirring motor and was thermostatically controlled to within 0.1° C. The water was usually kept at a moderately high temperature of about 26° to 30° C., since it had been noticed on several occasions that bleeding was inhibited at lower temperatures. Kramer (1940) had in fact shown that the transpiration rate of sunflower and privet plants was reduced to 20-30 per cent of the rate at 25° C. when the soil was cooled to 2° C. Although, in general, plants bled no better when in culture solution than in sand, it was hoped that this method would give a more exact comparability among the plants of any one experiment.

In order to roughly determine whether aeration of the nutrient during growth was beneficial to root growth and to the subsequent bleeding rates, four tomato plants were grown in gallon jars containing Hoagland's nutrient solution. The solution in two jars was aerated by means of sintered glass blowers, whereas the other two were not aerated. At the end of two weeks the roots were measured, the plants were decapitated at 15:00, and 6 mm. i.d. translucent rubber tubing attached to the stumps. The tubing was filled with distilled water to a central mark, so that either back suction or bleeding could be determined by the rate of change of the column height. This is shown in Table IV, where the rate is indicated at the mid-point of several time intervals. The advantage gained by aeration

TABLE IV

Bleeding rate of Aerated and Non-aerated Plants.

	Not Aerated		Aerated	
Plant number	1	2	3	4
Root length, cm.	21	23	39	45
Time	Bleeding rate (Change in Column ht., mm/hr)			
15:15	-8.0	-6.0	+4.0	+36.0
17.45	-1.5	-1.1	+2.7	+ 4.9
21.45	-1.7	-1.4	+2.0	+ 5.7
03:30	-0.9	-1.1	+1.1	(Leaked)
14:45	+0.4	+1.0	+1.5	+ 2.6
03:45	-0.6	-1.3	-0.7	- 0.6
22:15	-0.3	-0.9	-0.3	- 0.3

was obvious. Aeration systems were set up in both the large growing tanks and the bleeding trough, and used thereafter unless otherwise specified.

Pasadena city water varies considerably during the year as to total mineral content and to added chlorine. When it consists of almost pure Colorado River water, the solid content runs about 800 ppm, but when diluted with local well water, as it sometimes is, the content is considerably lower. The fact that the plants appear less vigorous and somewhat chlorotic at certain times of the year, in spite of careful control of pH of the nutrient and added iron, suggested that the tap water may be toxic, especially with respect to chlorine. To find if this were true, and also if any addendum to the nutrient would be effective in overcoming this toxicity, an experiment was set up involving different treatments of the nutrient. All solutions were 100 per cent Hoagland's, were maintained at pH 5.3 and had additional iron added once a week, unless otherwise specified. After two weeks the plants were decapitated and bled, the exudate being collected and measured over five different periods. The results will not be presented in detail, but are summarized in the following points:

1. Nutrient made of distilled water gave somewhat better bleeding than that made from tap water.
2. If pH were not constantly adjusted, it slowly rose to 7.0 or over and resulted in slightly chlorotic plants, but the bleeding of such plants was not inhibited.

3. Failure to add extra iron did not reduce growth or cause chlorosis over the two week period of the experiment, and bleeding was only very slightly reduced. Apparently an acidic pH is far more important for absorption of the iron ion than having a high concentration of the iron salt present.
4. Aeration again proved beneficial.
5. Boiling of tap water to remove dissolved gases aided somewhat. The plants bled about as well as when distilled water was used for the nutrient.
6. Addition of carbon black as an adsorbent proved exceedingly toxic. Plants became chlorotic and did not grow well. They bled only about one-third normal.
7. Variation of magnesium sulphate, calcium nitrate and potassium nitrate individually from 25 per cent to 200 per cent of the amount called for in normal Hoagland's solution failed to cause any very significant differences in bleeding, although conclusions should not be drawn on this point until more plants are used.
8. Pfeffer's solution gave results not unlike Hoagland's.

Guided by these findings, all successive plants were grown under conditions of aeration, careful pH control, and utilization of distilled or deionized water whenever any

evidence of toxicity was found in the tap water.

It seemed probable that plants partially starved by being placed in either CO_2 free air or darkness for a length of time would be more sensitive to externally applied sucrose. Consequently twelve plants were selected and placed in darkness at 30° C. for 48 hours. At the end of this period they were decapitated and had either seven per cent sucrose or water applied to two leaves. Not only did the sucrose-fed plants fail to bleed more than the controls as found by Dubnoff, but only one out of the entire twelve plants bled at all, that being one of the controls which bled only very feebly.

When a low carbohydrate plant failed to show a bleeding response, as just described, it was then considered whether or not one excessively high in assimilates would be any less sensitive to added sucrose, as one would teleologically believe. Four days before the experiment, the plants were placed under continuous artificial (fluorescent) illumination of 400 foot candle intensity, at 22° C. Upon decapitation and bleeding, there was found to be a sharp increase in the rate of bleeding of the sucrose-fed plants about 8 hours after application of the carbohydrate. This response was slightly faster than would normally be expected at 22° C., but showed undeniably that the reception of the bleeding stimulus by the roots is independent of the degree of previous assimilation, providing that reserves are not

completely depleted. The experiment strongly suggests that the mechanism of translocation operating in this case was completely independent of concentration gradient. Sucrose analysis of roots at the end of 20 hours of bleeding indicated 5.2 mg. per gram of dry weight for the sucrose-fed plants, and 4.2 mg. for the controls, a hardly significant difference.

Previous experiments had demonstrated that a sucrose solution of anywhere between 5 and 10 per cent applied to two leaves gave excellent bleeding increments over controls. In an effort to find the optimum concentration of sucrose, an experiment was carried out utilizing an 18.7 per cent solution of this sugar, actually a hypertonic concentration. Twelve plants were set up on the bleedometer in the normal manner, as previously described. An 8° C. cooling collar was applied to each of the first four plants, and 24° collars to the second set of four, both sets receiving the concentrated sucrose solution through two leaves on each plant. The third set, the control plants, simply had water applied to the leaves. When the bleeding rate of the sucrose-fed plants was plotted as a percentage of the bleeding rate of the controls, there was no inflection at all in case of the 24° C. plants, and only a very gradual rise in the 8° C. plants. This relationship would seem to indicate that plasmolysis of the leaf cells, caused by the concentrated sucrose solution, resulted in a decreased uptake and translocation. Since Dubnoff (1944) had already shown that there

was no significant difference between the volume of exudate of tomato plants with leaves in 1 or 2 per cent sucrose and control plants, it was assumed that 5 to 10 per cent was about the optimum range of concentration. Seven per cent was used in most succeeding experiments.

In order to determine whether other sugars were as effective as sucrose in promoting the bleeding stimulus, an experiment was undertaken utilizing 3.7 per cent glucose and 3.7 per cent mannitol. These concentrations were used because they had the same osmotic concentration as 7 per cent sucrose. Each solution was supplied to two leaves of two plants, the third pair of control plants having water supplied to the leaves. No stem cooling was used in this case, the plants being bled in the dark room, which was kept at 23° C. The sugars were applied at 11:00 and the bleeding rates recorded over 24 hours. Bleeding rates of the plants supplied glucose and mannitol were plotted as percentages of bleeding rates of the control plants, the curves being shown in Fig. 3. Also plotted in this graph for means of comparison, is the percentage curve common to sucrose, at the same temperature, this curve having been prepared from the means of the bleeding rates of about 200 plants. The first point of interest is the upward inflection of the mannitol curve at a time identical with that of the sucrose curve. Although mannitol may be utilized by Rhizobium as a source of carbon (Peterson and Peterson, 1945), and although it is found rather widely

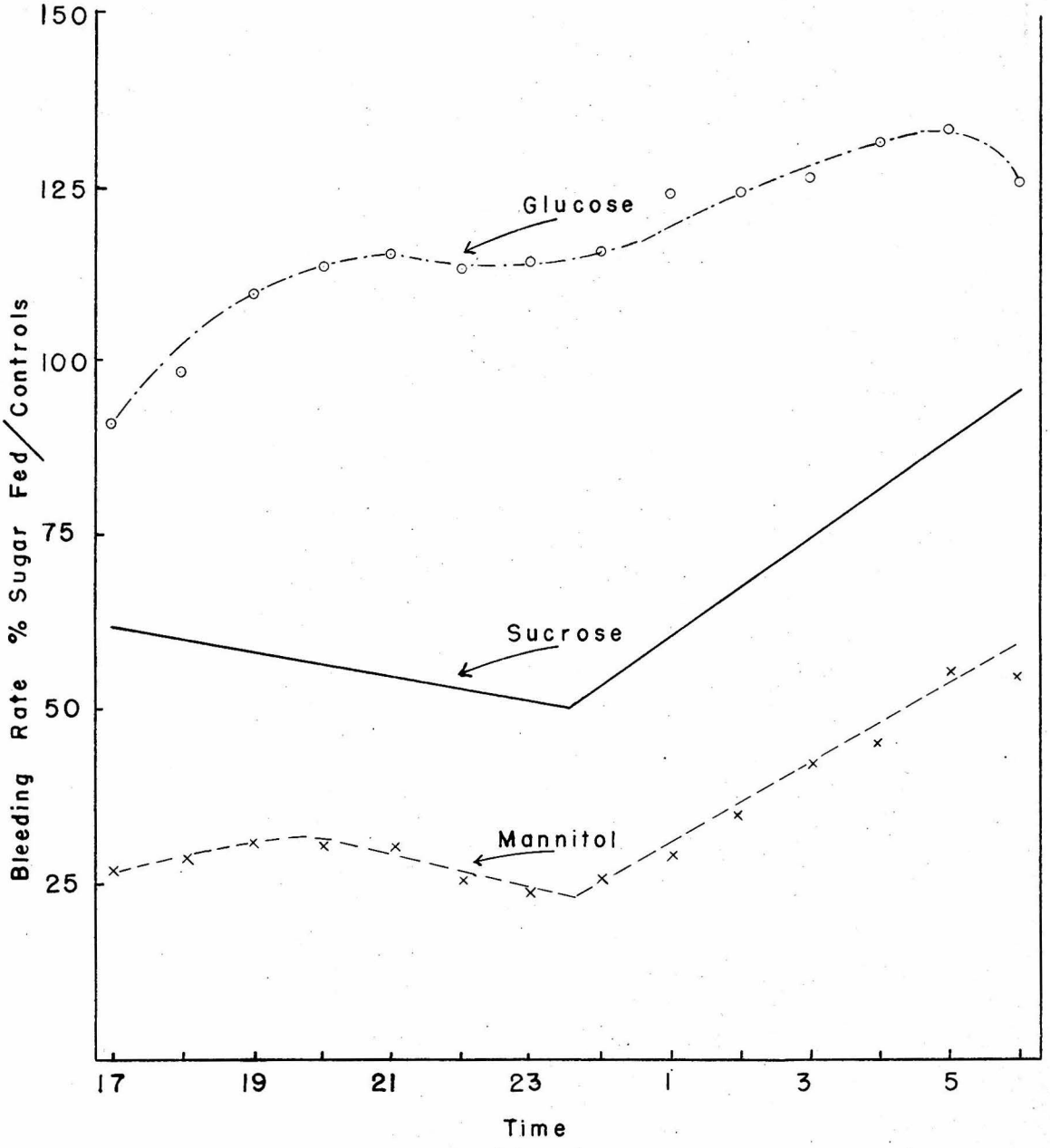


Fig. 3. Rate of bleeding of plants having two leaves in 3.7 per cent glucose or mannitol, or 7.0 per cent sucrose. The proportion of the rate of bleeding of each group of sugar fed plants in per cent of that of the water controls is plotted.

in higher plants, there is no evidence of its being utilized in metabolism by the latter. Judging from the curves, it appears evident that mannitol may be translocated by the same mechanism as sucrose, even though it may be a rather "foreign" substance to the tomato at such concentrations. If further experimentation with mannitol indicates this suggestion to be true, then the sugar may prove an ideal substance for the study of translocation, since, being metabolically inert, it would not be involved in respiratory losses. If it is truly inert, then the increased rate of bleeding which it produces must be ascribed only to an osmotic effect, the "vitalistic" theory previously discussed being inapplicable in this case. It is of course possible that mannitol, even though it be translocated to the root as such, increases the latter's metabolic activiity in a multi-fold manner, and that the increased bleeding noted in this case is simply a measure of the osmotic component. Interpretation of the glucose curve is more difficult. The fact that there is a constant rise almost from the time of application of the sugar, and that the final maximum reached is quite high, it would seem to indicate that the sugar is absorbed by the leaf and translocated very rapidly. Also, it may be utilized by the root <u>both</u> metabolically and osmotically in creating the very high bleeding rates. If absorption of sucrose does not occur as such, but only as its inversion products, as postulated by Said (1948), then the apparent rapid absorption rate of glucose as compared to

sucrose may simply be due to the fact that the former has no lag due to the inversion process. It should be mentioned that Went and Bonner (1943) found a solution of sucrose to be more active than glucose in causing growth of tomato plants in darkness, when applied to the leaves. Further experiments involving these and other sugars should be most enlightning.

At this point it appeared of interest to determine whether the 11 hour interval (at 23° C.) between application of the sugar and initial rise in bleeding rate held regardless of the time of day of application. Seven per cent sucrose was applied in the normal manner to three plants at 11:00, to three more at 16:00 and to the final set of three at 22:00. Three control plants had water applied at 11:00. The data indicated first of all, a sudden surge in the bleeding rate to about double its previous value, immediately after application of the sugar solution -- regardless of the time of application. This was apparently due to the rising sap, which had previously been partially lost by transpiration through the leaves, now being diverted to the stream exuding at the cut surface. Also, a small amount of water is absorbed by the leaves and probably adds to the bleeding stream. It was noted that there was the normal increment over control plants 11 hours after the sugar was applied at 11:00. When applied at 16:00 the response was very weak and seemed to come after only 6 hours, whereas with the 22:00 application, no response was exhibited. Thus, application

was most effective if timed so that the response came during the evening hours or period of minimum diurnal bleeding.

Since it was deemed desirable to study the effect of low temperatures upon the absorption of sucrose by leaves, several experiments were run in which the sucrose solution surrounding the leaves, as well as the stems, were cooled. In the first run, two plants had the stems cooled to 5° C. and the 7 per cent sucrose solutions about the leaves maintained at 10° C. by means of cold water circulating through small diameter rubber tubing which was coiled in the solutions. Two other plants simply had sucrose applied to the leaves, and the final two acted as controls with water about the leaves. Dark room temperature was 24° C. The bleeding data showed an increase over the controls 7 hours after sucrose application for the cooled plants and 10 hours after application for the plants at room temperature. Considering the inherent variation among individual plants, 7 hours is hardly significantly shorter than 8.5 hours, which is the mean for sucrose fed plants with the stems only cooled to 5° C. A second experiment was run in the same manner except that four plants were used in each group, the stems being cooled to 1° C. this time and the leaves to 8° C. Again there was an upward inflection of the bleeding percentage curve somewhat short of the mean time for plants with stems only cooled to 1° C. The inflection came after 5 hours compared to a mean of 8 hours at 1° C. Sucrose analysis

of the roots at the end of the bleeding failed to show a significant difference between any of the 3 groups. Thus we can say that cooling the surrounding solution and the leaves does not hinder, and probably slightly enhances absorption by the leaves and translocation of carbohydrates within the rachis.

In order to further determine which process took the greater amount of time, absorption by the leaves or translocation down the petioles and stems to the roots, the following experiment was designed. Seven per cent sucrose was applied to nine plants at 13:00, all plants being decapitated and connected to the bleedometer. Of these plants, three were left intact, two had the petioles of the two leaves submerged in the solution tightly pinched by means of screw clamps at 17:00, two more were so treated at 19:00, and the final pair were clamped at 21:00. Three plants were used as controls with leaves in water, and petioles left intact. Between 02:00 and 06:00 the next morning all of the sucrose-fed plants which had clamped petioles failed to show an increase in bleeding rate over the controls. Those in which the petioles were not pinched showed the normal increase over controls. The relationship is perhaps more readily apparent if the average absolute bleeding rate of each set of plants is indicated at 02:00 (just when the sugar effect was beginning to take place) and at 06:00 (when the effect has reached its maximum), as shown in Table V.

TABLE V

Bleeding Rate in Drops per Hour of Plants Fed Sucrose at 13:00, and Petioles Clamped at Various Intervals as Indicated.

Treatment	Time of Measurement		Change
	02:00	06:00	
Not Clamped	0.2	0.4	+0.2
Clamped 21:00	1.5	1.2	-0.3
Clamped 19:00	1.9	1.5	-0.4
Clamped 17:00	0.5	0.3	-0.2

In this experiment it was assumed that if translocation had proceeded into the stem at the time of clamping the petioles, there may be a sufficient amount accumulated in the stem to pass on down and increase the metabolic activity of the root, resulting in an increased bleeding. The fact that none of the plants with clamped petioles increased in bleeding rate between the hours of 02:00 and 06:00 probably indicates that absorption by the leaf and translocation down the rachis and petiole takes considerably longer than movement down the stem to the roots. No correlation was noted between the different times of clamping.

In order to determine whether translocation was cyanide inhibited, and consequently completely dependent upon the metabolic activity of living cells, a 3 cm. ring of NaCN in agar was applied around the stem of tomato plants, near

the base. Nine plants received sucrose through the leaves at 12:00 and three received water. Of the nine, three had 0.25 M NaCN applied, three had 0.0025 M NaCN, and the third set had plain agar. The last set of three with leaves in water served as controls. As seen in Fig. 4, the bleeding rate of the plain agar ringed plants with respect to the controls, began increasing about 23:00. The plants with the lower concentration of cyanide, although bleeding at almost the same rate as the plain agar plants at 18:00, at first began to decrease, but finally reached a maximum at 07:00 about half as great as the plain agar plants. Plants with the high concentration of 0.25 M showed no response at all, and constantly decreased while the other plants were increasing. At about 06:00, however, they did begin to recover, and by 13:30 had reached a rate equal to that of the plants not given cyanide. The higher concentration of cyanide visibly injured the stem, as did an equivalent molar concentration of sodium chloride in agar, even though such concentrations are osmotically hypotonic to the stem tissue. It is probable that the phloem was completely killed by the higher cyanide concentration, and consequently the fact that the bleeding recovered and became equal to that of the untreated plants would indicate that although the narcotized phloem effectively inhibited the downward translocation of organic materials, the xylem, which undoubtedly received a fair dose of cyanide by diffusion across the

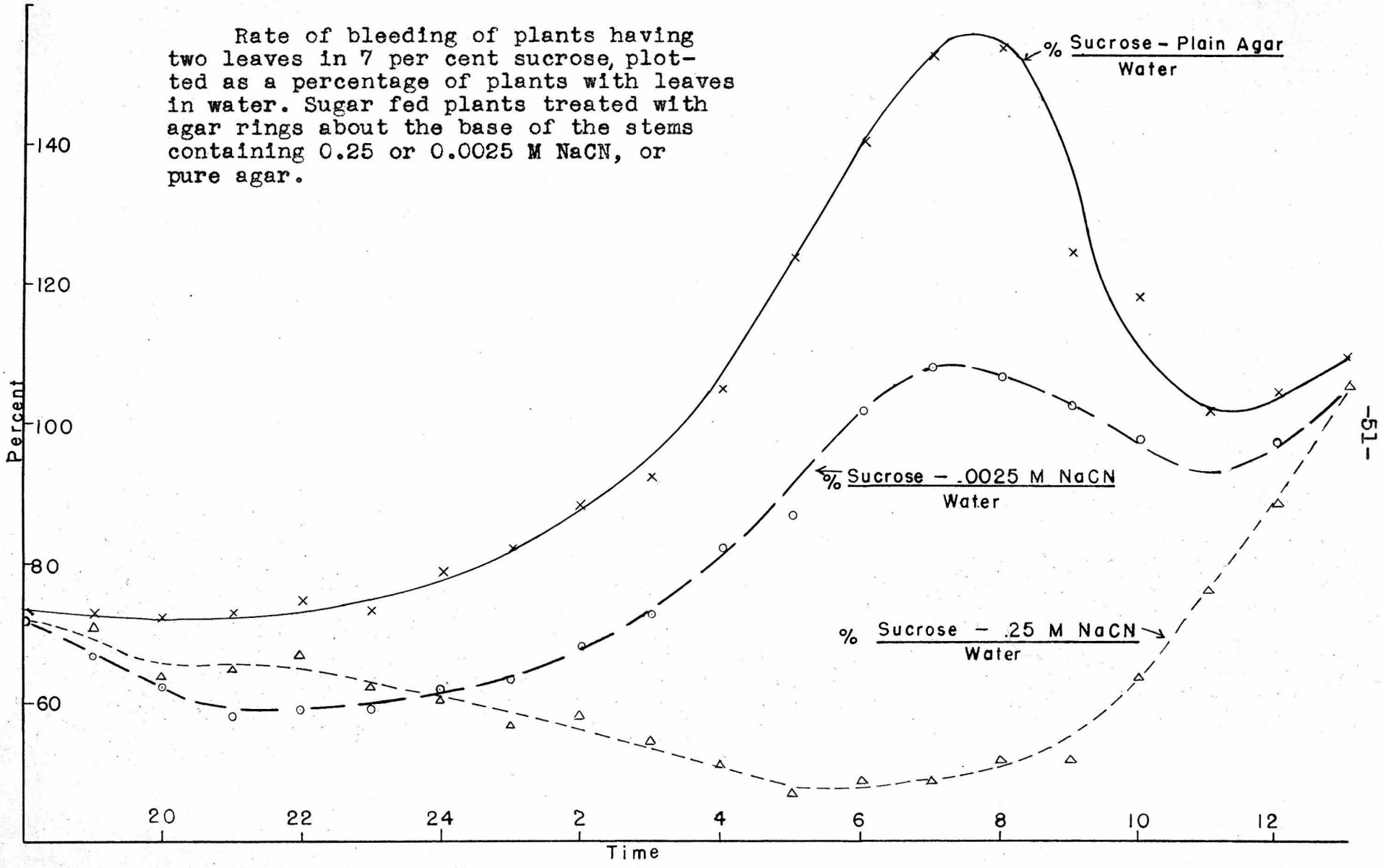

Rate of bleeding of plants having two leaves in 7 per cent sucrose, plotted as a percentage of plants with leaves in water. Sugar fed plants treated with agar rings about the base of the stems containing 0.25 or 0.0025 M NaCN, or pure agar.
Percent
140
120
100
80
60
% Sucrose - Plain Agar / Water
% Sucrose - .0025 M NaCN / Water
% Sucrose - .25 M NaCN / Water
20
22
24
2
4
6
8
10
12
Time

Fig. 4

cambium, did not suppress the upward flow of sap. The fact that the sucrose effect was completely abolished in the case of the higher concentration would therefore indicate the necessity of living tissue for realization of this effect. These findings are in line with the previously mentioned work of Heyl (1933) in which the bleeding sap was suppressed by hydrogen or certain narcotics, and also of Speidel (1939) who found that bleeding was decreased by the addition of phenylurethane to the nutrient medium or by decreasing the available oxygen supply. He also noted that potassium decreased the bleeding rate, whereas calcium increased it. These addenda, however, were all in the nutrient solution, and therefore are not comparable with the author's experiments in which application was around the base of the stem.

Since future experiments were planned utilizing sucrose isotopically tagged with C^{14}, it was deemed necessary to find the most efficient method of application to the leaves. Obviously, because of the expense of the isotope, a large volume of solution of which only a minute amount would be absorbed was out of the question. To test the absorption from a small aliquot, ordinary sucrose was made up in a solution of 10 per cent concentration in 1 per cent agar. On the first four plants, 1 cc. was applied on both sides to each of the five leaflets of two leaves, a total of 10 cc. per plant. The second set of four plants had 1 cc. applied only to the terminal leaflet of each of the two leaves or 2 cc. per plant. The four control plants had application

similar to the latter, minus the sucrose. Bleeding data indicated a behavior almost identical as if the leaves had been in a sucrose solution. Both the first and second sets of plants showed an increased rate of bleeding over the controls at the end of 11 hours, the normal time for a temperature of 20° C., at which the room was maintained. The final maximum was slightly greater in case of the plants supplied with 10 cc., as would be expected. This experiment also indicates that the rate of absorption and/or translocation is independent of the turgor of the leaf and possibly the rachis and petiole cells, the tissues almost surely being under a higher turgor when submerged in a solution which is actively absorbed. The agar soon became dry and could not contribute much to the leaf turgor. Also, the fact that different concentrations of sucrose (5, 7, and 10 per cent), each of which would give a different turgor pressure within the leaf, all result in about the same translocation rate as determined by the bleeding process, again suggests that turgor, within certain limits, is not a factor in translocation rate. This has been noted by Dijkstra (1937).

A similar experiment was performed in which a 7 per cent sucrose solution in 0.8 per cent Drene (a detergent to cause better adherence of the solution) was painted directly onto the leaves. The first group of three plants had just the lower surfaces of the two leaves painted, while the second group had both surfaces painted. Controls were thus supplied with detergent solution only. Although the plants did not

bleed a great deal due to the high foliar transpiration, the data showed an increased bleeding rate after 11 hours in the case of the plants with both surfaces of their leaves covered, thus indicating the method to be successful. This was the method later adopted for application of C^{14} sucrose to the leaves. The fact that Went and Carter (1948) have shown marked growth responses of tomato plants by either dipping leaves into or spraying the foliage with a 10 per cent sucrose solution also substantiates the effectiveness of such a method of application.

A preliminary bleeding experiment was performed using squash, Cucurbita pepo, and was set up just as in the case of tomatoes. Two leaves were left on each plant, just below the decapitated stump, and were immersed in the solution. Below these leaves the cooling collar was applied, being 1° C. in this experiment. Four plants had 7 per cent sucrose and 1° C. collars, the second set of four had the sucrose and 23° C. collars, and the final four were controls with leaves in water and 23° C. collars. Ten of the twelve plants bled for only 9 hours or less, but two bled for over 56 hours, one of these being a cooled-jacket plant and the other being a control. The control plants bled from 2 to 12 times faster than any of the sugar-fed plants for the first 2 to 3 hours, but rapidly decreased in rate, so that by the end of 7 hours (now considering only the two plants which bled well) the rates had reversed, and the 1° C. sucrose-fed plant was bleeding faster than the

control. There was no sudden upward inflection in the percentage curve, but only a general rise beginning right after sugar application -- not unlike the experiment in which glucose was applied to tomato plants. At any rate, it was deemed that squash was a less satisfactory plant for bleeding experimentation than tomato, and no further work was done with it. Also, it was a rather difficult plant to properly set up on the bleedometer.

In a preliminary experiment to check the possibility of different amounts of carbohydrate in the exudate being dependent upon treatment, twelve tomato plants were set up in two groups of six plants each. One group had 7 per cent sucrose supplied to the leaves, and the other had water. The groups were further subdivided into 1° C. stems and 20° C. stems, each sub-group containing three plants. Sucrose or water was applied at 13:00, the plants decapitated, and the exudate collected and measured over three following periods: 16:00 - 19:30, 19:30 - 10:00, and 10:00 - 17:30. Volumes showed the general trend previously described by the more accurate bleedometer recordings. Sucrose analyses were made on exudates from the middle period for each of the four groups of plants. The values obtained, however, were exceedingly low, ranging from 0.000 to 0.013 per cent. Reducing power before inversion was even much lower than the increment due to inversion, and consequently reducing sugars were utterly nonexistent in the exudate. No general trend was noted.

It seemed possible that if the exudate collection were fractionated into 10 short periods, commencing immediately after decapitation, a slight increment in the sugar concentration of the exudate which might be associated with the increased activity of the root system at the 8 to 11 hour period, might be picked up. The design of the experiment was identical to the one last described except that the plants were decapitated at 10:15, right after arrangement of the cooling collars, and the sucrose was not supplied until 13:00. This was done so that in case of any initial increment in sucrose content of the exudate, it could be attributed to an artifact caused by the cutting, and not to addition of the sugar. The first sample was collected immediately following decapitation, all periods being indicated in Table VI. The volume of exudate flow per hour is indicated for each period and group of plants as is the sucrose content in per cent, these data being derived from the exudate of all three plants in each group lumped together. Just as in the last experiment, the sucrose values are exceedingly low. The accuracy of the analysis at this concentration runs about $\pm$ 0.0005 per cent, and thus some of the smaller fluctuations are not significant. The higher concentration found in the first sample of three of the groups is significant, however, and is most likely due to a sudden release of the high-sugar content phloem sap at the cut surface, or of the contents of the wounded cells, with consequent contamination of the transpiration stream rising

TABLE VI

Bleeding Rates and Sucrose Concentration of Exudates Over Several Periods of Time of Tomato Plants Supplied Sucrose or Water Through the Leaves and With Stems Maintained at 1° C. or 20° C.

Period of Collection	Stems at 1° C.				Stems at 20° C.			
	Sucrose		Water		Sucrose		Water	
	Rate cc/hr	Conc. %	Rate cc/hr	Conc. %	Rate cc/hr	Conc. %	Rate cc/hr	Conc. %
10:15-11:15	5.9	0.0009	4.1	0.0034	4.6	0.0021	3.9	0.0024
11:15-14:45	3.1	0.0003	2.7	0.0011	2.9	0.0008	3.3	0.0027
14:45-18:45	2.4	0.0008	1.8	0.0018	2.0	0.0014	2.1	0.0011
18:45-22:45	1.8	0.0008	1.4	0.0018	1.1	0.0018	1.5	0.0011
22:45-02:45	1.7	0.0006	1.4	0.0009	1.0	0.0011	1.3	0.0008
02:45-07:45	1.5+	0.0008	1.3+	0.0012	1.2+	0.0011	1.1+	0.0000
07:45-12:45	1.7	0.0005	1.3	0.0009	2.1	0.0008	1.2	0.0006
12:45-18:45	1.1	0.0006	0.8	0.0009	1.0	0.0012	0.8	0.0006
18:45-23:15	0.9	0.0008	0.6	0.0014	0.9	0.0017	0.7	0.0015
23:15-08:15	0.8	0.0008	0.6	0.0018	0.7	0.0011	0.5	0.0017

through the xylem. In none of the plants was there a significant increase in concentration at the 8 to 11 hour period, or at any other period other than the first one. Reducing sugars, along with other reducing substances, had a value considerably lower than sucrose, and did not show as great an increase at the first period. It would seem that the increased bleeding rate due to sugar application is mediated

through active metabolism of the root cells, but in such a way that all carbohydrates are retained within the cell or lost through respiration, and not released to the transpiration stream. The above findings essentially confirm the work of vanOverbeek (1942), in which he noted that the first sample of exudate collected from decapitated tomatoes contained never over 0.01 per cent of sucrose and glucose, whereas later samples seemingly contained no sugar. Chemical analysis of the exudate from Cucurbita pepo have been made by Litvinov (1927). He also found no sugar, but small amounts of plant acids, albumens, amino acids, nitrates, nitrites, and certain other inorganic ions. Dry residue and ash had a value of 2.6 and 1.1 grams per litre of exudate respectively.

In all of the previously described experiments, the bleeding response was mediated through externally applied carbohydrate. In an effort to determine if the response could also be caused by the naturally formed assimilate of the plant, a series of experiments was planned in which light was to be the activating mechanism, thus causing the plant to manufacture its own carbohydrate. If different intensities of reaction or different time intervals between application and reaction were noted when compared to similar values of experiments involving sucrose-feeding, some light may be shed upon rates of absorption within the leaf, the most common form of sugar translocated, etc. Would one

expect the externally applied sugar which had to travel largely through stomata, intercellular spaces, vascular sheaths, and finally into the phloem of the leaf veins, to reach the petiole faster than natural carbohydrate photosynthetically elaborated within the parenchyma cells? Perhaps this type of experiment would help answer these problems.

Initially, twelve San Jose Canner plants which had been grown in 6-inch pots with a 50-50 mixture of vermiculite and gravel, were brought to the bleeding room the evening before the experiment. In an attempt to prevent transpiration from the leaves which may cause failure to bleed, the room was humidified to a relative humidity of 75-82 per cent, the temperature being 24.5° C. ± 2.0° C. The plants were divided into two groups, those with collars at 2° C., and those with collars at room temperature. Half of each of these groups (3 plants) had the base of the stem girdled by application of steam jets for three minutes. Upon decapitation and illumination at 11:00, the plants failed to bleed. At 15:30, still not bleeding, the petioles were all tightly pinched with screw clamps, but this treatment did not induce bleeding. At 16:30 the lights were extinguished, and at 22:30 the plants were harvested for sugar analyses, the three individuals of each group being lumped together. Stem sections of 1.5 cm. length,

immediately above the girdle, or at corresponding positions on non-girdled plants, were analyzed, as were roots. The data are indicated in Table VII, and are discussed later.

TABLE VII

Sucrose Content in mg. per g. Dry Weight of Stem Sections above Girdle, or of Corresponding Sections on Ungirdled Plants, and of Roots.

Treatment	1.5 cm. section above girdle	Roots
2° C. Stem, Girdled	0.98	0.40
2° C. Stem, Not Girdled	0.64	1.48
24.5° C. Stem, Girdled	1.41	1.21
24.5° C. Stem, Not Girdled	1.09	2.43

A similar experiment to the above was set up, except that all of the leaves were placed within tightly closed clear plastic bags which had previously been wetted inside to help maintain a virtually saturated atmosphere within. Lights were turned on at 12:00 and the bags were supplied with CO_2 every few hours. Although a preliminary experiment on one plant indicated fairly good bleeding when the leaves were thus enclosed, only two of the twelve plants bled for more than several hours. At any rate, lights were extinguished at 18:30, and the plants were left connected to the bleedometer until 11:00 the next day, at which time they were harvested. Since the two plants which bled were both in one group (24.5° C. stem, not girdled), no conclusions

could be gleaned from the bleeding data. Sucrose analysis, just as in the last experiment, was made of stem sections and roots, and is presented in Table VIII. Although the two experiments described are not comparable as to sucrose

TABLE VIII

Sucrose content in mg. per g. Dry Weight of Stem sections above Girdle, or of Corresponding Sections on Non-girdled Plants, and of Roots.

Treatment	1.5 cm. section above girdle	Roots
2° C. Stem, Girdled	1.57	1.08
2° C. Stem, Not Girdled	1.10	1.62
24.5° C. Stem, Girdled	1.13	1.08
24.5° C. Stem, Not Girdled	1.64	1.62

content, since the latter one was harvested the following morning, and did not have the petioles clamped but did have leaves in plastic bags, there nevertheless are some deductions which may be made from both experiments:

1. The roots are higher in sucrose in every case where the plants are not girdled, regardless of stem temperature. It is interesting to note that the reducing power of the roots between any comparable group of girdled and non-girdled plants never differed by over 5 per cent, whereas the sucrose values, as seen from the above tables, differed by up to almost 300 per cent. This clearly demonstrates the effectiveness of the steam-girdles in preventing translocation to the roots, and also suggests that carbohydrate

is translocated almost entirely as sucrose, or else undergoes conversion to this form within a very short time after arrival in the root.

2. When the stem sections are considered it appears that in three out of the four possible comparisons between girdled and non-girdled plants, regardless of stem temperature, a 40 to 50 per cent greater accumulation of sucrose was found above the girdle as compared to corresponding sections on non-girdled plants. Reducing power in all four of these comparisons was greater in the stems of non-girdled plants, but only to a very slight amount of about 6 per cent, thus strongly indicating transport in the form of sucrose.

3. It should be emphasized that all of these carbohydrate values are exceedingly low, and that a difference of only one drop in the titration could cause a reversed relation of sucrose concentration in any two comparable groups of girdled and non-girdled plants.

4. The data do not appear to include a sufficient number of plants to draw statistical conclusions regarding temperature effect.

In an effort to give plants light, and still have them bleed, it was decided that the light would have to be given first, and then the stem decapitated below all of the leaves, thus preventing robbery of the bleeding stream by foliar transpiration. This method was not considered ideal, since the exact time of arrival of the carbohydrate into the stem was not known, and its arrival, of necessity, should be

before decapitation. In order to determine whether such a method would be feasible, two tomato plants were removed to the dark bleeding room in the evening. The following morning at 08:15 one was exposed to fluorescent light, the other being left in the dark at the same temperature (23° C.). At 12:00 both plants were decapitated below the three upper mature leaves, which were the only ones that had been left on the plants, and connected to the bleedometer. The plant given light started out bleeding at about twice the rate of the darkened plant, and at the end of 8 hours was bleeding over four times as fast. The datum is suggestive of a rather rapid translocation rate from leaf to root, but of course is not significant with just the two plants.

An enlargement of the above experiment was planned, in which the plants would be subjected to different lengths of photoperiod before decapitation and bleeding. Twelve San Jose Canner tomato plants, all about 60 cm. in height, were selected and taken to the bleeding room the evening before the experiment. The plants had previously had all of the lower leaves removed, leaving only the upper four or five mature leaves present, which were of approximately an equivalent area in each plant. At 09:00 four plants were placed under fluorescent illumination (500 foot candle) at 24° C., the other eight remaining in the dark room at the same temperature. At 14:30 four more were removed to the lights, and at 20:00 the lights were extinguished and

the plants decapitated and bled. Thus the three groups received an 11 and a 5½ hour photoperiod, and no light respectively. Two leaves were left on the stems just below the point of decapitation in order not to completely deplete the plants of a reservoir of carbohydrate. When the plants are not illuminated, transpiration appears to be sufficiently reduced so that bleeding still takes place in spite of the leaves remaining on the stump. All twelve plants bled very well, some of them up to five days. The bleeding rates of all three groups are plotted in Fig. 5, as are the percentage values of 11 hour photoperiod/dark, and 5½ hour photoperiod/dark. The striking manner in which the long-photoperiod plants bled at such a high rate immediately after decapitation is of interest. Although the peak of the curve is at 02:00, we have no way of telling when the increment first began, since the plants were still intact at the time when it probably would have occurred. Since, in this group of plants, light was first given at 09:00, we can say that translocation to the roots occurred between the hours of 09:00 and 02:00, or within 17 hours. Considering that the curve may begin to increase many hours before 02:00, and that there must be a considerable lag between formation of the first photosynthetic assimilates in the leaf parenchyma and their transport in significant quantity to the sieve tubes, it appears likely that the actual transport occurred in far less than 17 hours. On the other hand, upon consideration of the plants which received only a 5½ hour photoperiod,

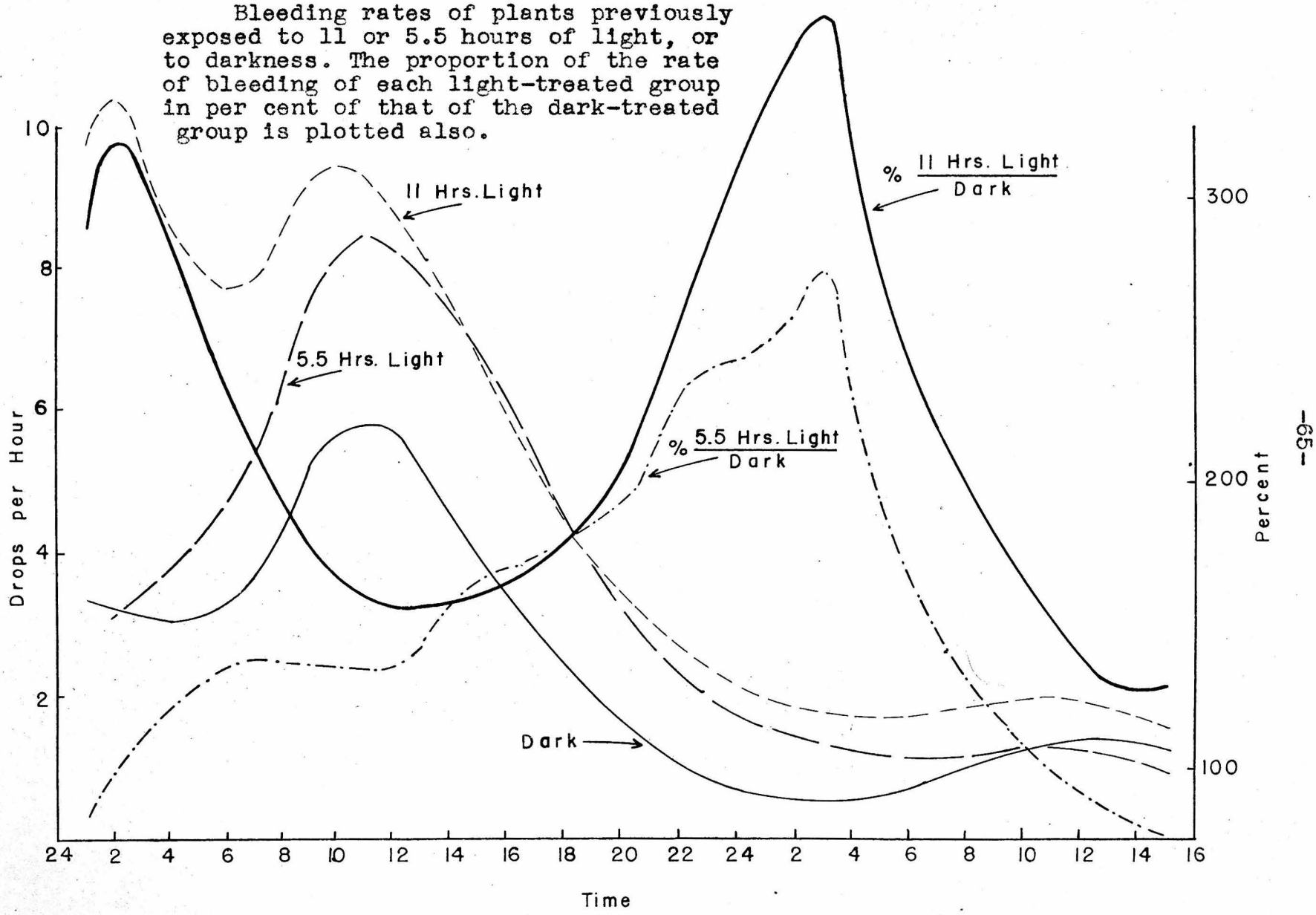

Bleeding rates of plants previously exposed to 11 or 5.5 hours of light, or to darkness. The proportion of the rate of bleeding of each light-treated group in per cent of that of the dark-treated group is plotted also.
11 Hrs. Light
5.5 Hrs. Light
Dark
% 11 Hrs. Light / Dark
% 5.5 Hrs. Light / Dark
Drops per Hour
Percent
Time
10
8
6
4
2
300
200
100
24 2 4 6 8 10 12 14 16 18 20 22 24 2 4 6 8 10 12 14 16

Fig. 5

we see that the increase over controls (dark plants) did not begin to take place until after 02:00. Since these plants first received light at 14:30, this would imply that translocation took place between these two periods, or within 11½ hours. This interval would be shortened only by subtraction of the above-mentioned lag due to movement from parenchyma to sieve tubes. Another point of interest, in the case of the longer-photoperiod plants, is the second and even greater peak occurring at 03:00 on the following day. It would seem to represent a second surge of carbohydrates to the root, completely separate and distinct from the first movement. These two separate increments in bleeding rate, as compared to the darkened plants, apparently arose from assimilates elucidated by one and the same photoperiod-the only one given. If this interpretation is true, it is strongly suggestive that either maximum transport occurs in cycles, only at certain period of the day, or that it occurs more or less constantly once the assimilate is formed, but is activated by two distinct mechanisms, each operating at its own velocity. The peaks can not be ascribed to change in type of carbohydrate present or other factors caused by temperature alterations, because temperature is held constant once bleeding is begun.

It has been noticed on several occasions that very large plants invariably bled very strongly, as would be expected from a well-developed root system. It was considered possible that the transpiration stream of such

plants may have a sufficient volume of flow to overcome the transpiration of two leaves left on the plant, even when the leaves were brightly illuminated. Two such plants (1 m. tall) were brought to the darkened bleeding room one evening and were decapitated the next morning at 09:00. After 23 hours of intense and almost equal bleeding in the dark, one of the plants was illuminated with both fluorescent and Mazda lighting. The latter apparently gave off such intense infra-red radiation that the heating effect on the leaves rapidly increased transpiration, resulting almost in cessation of bleeding. Upon observation of this effect, about two hours after illumination was begun, the Mazda was turned off. The fluorescents were extinguished at 13:30, having been on over five hours. Although the bleeding rate of the illuminated plant was slightly less than the darkened one while the lights were on, due probably entirely to increased transpiration, the rate came up to that of the darkened plant by 17:00. By 02:00 the next morning the bleeding rate of the illuminated plant had risen to .5 times that of the darkened one, the increased rate first becoming apparent between the hours of 20:00 and 22:00. This again indicates a translocation rate of about 12 hours from leaf to root. It also, along with the last experiment, directs attention to the somewhat greater root activity that may be achieved through the action of naturally formed assimilate as compared to artificially supplied carbohydrate.

These experiments in bleeding have dealt only with a relatively narrow range of methods and materials -- several

of the carbohydrates. That there are other substances of great importance synthesized and utilized in the plant, and which are important in the processes of absorption and translocation is not denied. For example, Skoog (1938) has shown that application of indole-3-acetic acid (10 mg./g. of lanolin) to the upper part of a decapitated etiolated Pisum shoot or to a decapitated Helianthus shoot caused about a 4X increase in volume of exudation during the next 160 hours. The auxin was considerably more effective in increasing exudation if applied near the upper part of the decapitated shoot, making it hard to visualize its effect on exudation acting via translocation to the root. The ineffectiveness of basal application may of course have been due to a lower sensitivity or absorptive power of the more mature basal tissue. The fact that a large increase in exudation was found only when application was near the tip, regardless of whether the shoot was cut short or long, may indicate that the influence of lateral buds above the point of application has an inhibitory effect. Such responses in both species of plants are dependent upon the presence of the attached seed or upon previous illumination, thus demonstrating the requirement of stored material for continued exudation. It is probable that the influence of auxin is related to utilization of this storage material. Dubnoff (1944) has in fact shown that tomato plants supplied indole-3-acetic acid to the roots and sucrose solution to the leaves, bleed more than plants supplied only the sucrose.

This increased bleeding rate was associated with a decreased sucrose concentration in the roots, due apparently to its increased utilization by the auxin.

It is realized that many of the bleeding experiments herein described are not directly related with temperature effect. A large percentage of them were performed in an effort to establish optimum conditions under which the final temperature experiments reported in the work of Went and Hull (1949) were carried out.

Miscellaneous Aspects of Translocation

In addition to the sucrose changes *in vivo* which external application of this sugar brings about, it was deemed advisable to learn what changes such application might have on certain other carbohydrates and plant acids.

The initial experiment was an analysis of stems and roots of tomato plants which had bled over night in the normal manner. Six plants were fed 7 per cent sucrose through the leaves, and six were given water. On the following morning they were harvested, immediately cut into stem sections (8 cm. sections taken mid-way between the roots and two feeding leaves) and roots, and dried in the forced draft oven at about 70° C. An 80 per cent ethanol extract was made on the dried, ground material, the six plants being lumped together in each case. The extract was cleared of non-sugar reducing substances, and was then used for the determination of fructose according to the colorimetric method of Roe (1934), and of reducing sugars according to the method of Hassid (1937) by titration with ceric sulphate. Glucose was roughly estimated by subtracting fructose from the reducing sugars. An acidified ether extract of the material was used for determination of total organic acids by titration with 0.05 N HNO_3 from pH 8.0 to pH 2.6, and for determination of oxalic acid by oxidation with permanganate. The methods used in the determination of these and other plant acids to be reported on,

were essentially methods developed by Vickery, Pucher, et al. (1934, 1941). The values found for these substances are shown in Table IX. Fructose and the plant

TABLE IX

Values of Certain Substances in mg. or m. Equivalent per gram of Dry Stem or Root Tissue After 24 Hours of Feeding 7 per cent Sucrose or Water to Two Leaves.

Leaf immersed in	Tissue analyzed	Fructose mg./g.	Glucose mg./g.	Total Acids m. eq./g	Oxalic Acid m. eq./g.
Sucrose	Stem	4.3	5.5	3.28	1.98
Water	Stem	4.5	4.3	3.17	2.03
Sucrose	Root	2.9	7.2	1.86	1.23
Water	Root	2.2	7.5	2.46	1.87

acids appear to be in higher concentration in the stems, and glucose higher in the roots, regardless of whether the plant was supplied sucrose or not. Differences between the tissues in the case of sucrose vs. water supplied leaves are probably not significant.

Since the organic acids of plants are important intermediates in carbohydrate metabolism, it was considered desirable to follow the path of isotopic C^{14} through the acids, and certain other fractions of the plant, when supplied to the leaves in the form of radioactive sucrose. Consequently several preliminary experiments were carried out to determine recovery of certain of the organic plant acids and carbohydrates.

To 2 g. of dried, ground tomato leaves were added small amounts of fructose, glucose, oxalic acid and citric acid, as indicated in Table X. This material and also 2 g. of the same dried tissue without addenda were each extracted first with acidified ether for the plant acids, and then with 80 per cent ethanol for the carbohydrates, after first neutralizing the tissue. Determinations were made as previously described, and in addition citric acid and l-malic acid were determined colorimetrically. All values are indicated in Table X.

TABLE X

Recovery of Various Addenda to 2-gram Samples of Dried Tomato Leaves.

	Carbohydrates (mg.)		Organic Acids (m. eq.)			
	Fructose	Reducing Sugar[1]	Total Acids	Oxalic Acid	Citric Acid	l-malic Acid
Amount added	5.0	15.0	1.245	0.635	0.312	0.298
Amount recovered	6.0	1.6	1.170	0.780	0.312	0.310
Percentage recovery	120	11	94	123	100	104

1. The reducing sugar consisted of 10 mg. glucose plus the 5 mg. fructose.

All recoveries were moderately good except reducing sugar. This was possibly due to a decomposition resulting from the strongly acidified condition of the residue from the ether extraction, and failure to immediately neutralize it.

The experiment was repeated, taking this precaution into consideration, and using this time sucrose, glucose and citric acid as addenda. Sucrose was determined by hydrolysis with HCl, and noting resultant increase in reducing power. Recoveries in this case were: sucrose, 91 per cent; glucose, 128 per cent; and citric acid, 80 per cent. In considering these percentage recoveries, it must be realized that the small addenda of 5-10 mg. was in each case only a minute percentage of the material already present in the tissue.

Since most plants that had been bled were harvested the morning after the experiment, when the sugar effect had largely disappeared, as explained in Went and Hull (1949), it was considered important to find what the distribution of sugar was within the plant at the time when the applied sucrose had caused most active bleeding. Four tomato plants 4 months of age and four more which were 2 3/4 months old were divided into four series — each series consisting of one old and one young plant. The four series included 7 per cent sucrose supplied to two leaves with stems at 1° C. and 21° C., and water supplied to two leaves with stems at the above temperatures. Bleeding was started at 12:00, however, only the older plants bled. At 01:00 the next morning, when the sugar-fed plants were bleeding far more rapidly than the controls, the plants were all harvested and immediately sectioned and dried in the forced draft oven. Sections included roots

together with the lower 3 cm. portion of the stem, and the upper portion which included the two petioles and the stem between the loci 1 cm. below the original decapitation and 1 cm. below the top of the cooling collar. Table XI indicates the sucrose values of these tissues for both age plants. Although the two different ages of plants are not strictly

TABLE XI

Sucrose Concentration (mg. per g. dry weight) of Tops and Roots of Tomato Plants Supplied Sucrose or Water through the Leaves, with Stems at either 1° C. or 21° C., harvested 13 Hours after Application.

	1° C.				21° C.			
	Sucrose		Water		Sucrose		Water	
Age (mos.)	4	2-3/4	4	2-3/4	4	2-3/4	4	2-3/4
Tops	2.38	2.68	2.68	0.89	5.51	5.06	2.38	1.78
Roots	2.53	2.08	3.57	1.78	3.28	3.13	5.21*	1.78

* Value probably high due to the small size of these roots.

comparable because of the fact that only one series bled, and because of the age difference itself, there are nevertheless some interesting conclusions which may be drawn from the results. The large accumulation of sucrose in the tops of the sucrose-fed plants with 21° C. stems suggests a blocking of downward translocation at that temperature. In both ages of sucrose-fed plants there is considerably less sucrose present in the tops of the cooled stem plants. The difference between sucrose concentration in

tops of sucrose vs. water-fed plants is much greater at 21° C. than at 1° C., showing that sucrose applied to the leaves accumulates scarcely more at the top of a cold stem than when just water is applied. The piling up of sucrose appears to be quite significant at the top of the 21° C. stems of the sugar-fed plants. When only water is applied to the leaves, regardless of stem temperature, the concentration of sugar is usually higher in the roots than in the tops, i.e. in three of the four cases above, the fourth being equal. However, when sucrose is applied, this relation is reduced to some extent for the 1° C. stems, but completely reversed in case of the 21° C. stems, again indicating a block by the warm stem.

In order to study the more detailed distribution of carbohydrates within the plant, the following experiment was designed. Twelve plants were set up on the bleedometer under exactly the same conditions as described in the last experiment, except that the stem temperatures were 1° C. and 20° C. Sucrose solution or water was applied to the leaves at 13:00, and the plants were allowed to bleed until 17:30 the following day, at which time they were dissected into five separate sections, as shown in Fig. 6 and immediately dried. The three plants of each series received identical treatment+were grouped for analysis.

Another experiment was run with exactly the same treatments, the only difference being that the plants had been transplanted slightly longer, and bled more vigorously

apparently due to a better developed root system. They were allowed to bleed over the second night, and harvested the following morning. In spite of these slightly different treatments, the results of both experiments are compared in Table XII.

TABLE XII

Sucrose Concentrations in mg. per g. Dry Weight of Different Sections Indicated in Fig. 6 for Plants Receiving 7 per cent Sucrose or Water through the Leaves, and with Stems at 1° C. or 20° C.

Data of Two Experiments Included.

	1° C.				20° C.			
	Sucrose		Water		Sucrose		Water	
Section	1	2	1	2	1	2	1	2
1	3.56	7.37	3.43	4.96	5.59	9.28	2.03	6.48
2	7.11	11.06	3.56	7.24	3.81	8.52	3.43	6.37
3	5.84	10.42	3.93	5.47	3.17	6.12	2.03	5.09
4	8.25	4.96	4.82	2.42	4.19	2.67	4.19	1.78
5	3.43	2.42	3.30	2.16	1.78	3.95	3.43	0.89

These concentrations again indicate a strong tendency for sucrose to accumulate at the top of the 20° C. stems when this sugar is externally applied to the leaves. There is also a high concentration within the stem (sections 2, 3, and 4) at the low stem temperature, but only when sucrose is applied to the leaves. This strongly indicates that the sucrose present in the 1° C. stem is the same sucrose which was applied to the leaves, and not simply a result of inter-conversion from some other carbohydrate as a result of low

temperature. The cold stem appeared to have almost no effect on any of the five sections as far as the plants supplied only water are concerned. The means of the two experiments are plotted in Fig. 7, and give a more clear concept of the relations.

It appeared advisable to make a more detailed carbohydrate analysis of the center sections of sucrose-fed 1° C. plant and the sucrose-fed 20° C. plant. In addition to sucrose, these sections were therefore analyzed for fructose and reducing sugars by methods previously described, and for starch by means of reducing power on a clarified extract hydrolyzed with takadiastase. Duplicate determinations were made on reducing sugars, and sucrose was determined by three different methods, all of which are indicated in Table XIII. Practically all carbohydrates

TABLE XIII

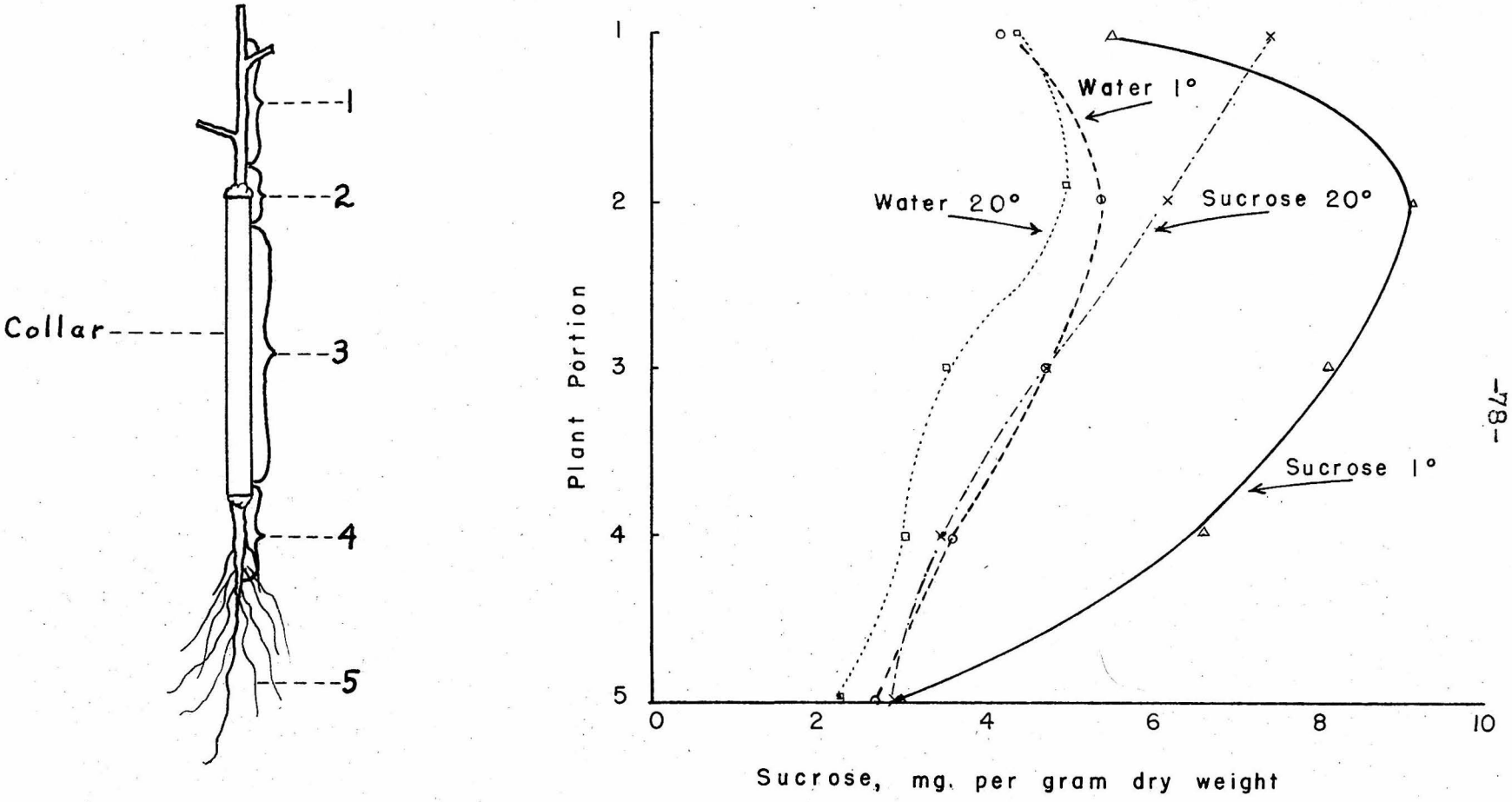
Carbohydrate Concentrations in mg. per g. dry weight of Stems within Collars Maintained at 1° C. or 20° C. All Plants fed 7 per cent Sucrose through two Leaves.

Sucrose values by three methods

Stem Temp. °C.	Water Extraction Invertase Hydrolysis	80 per cent Ethanol Extraction Invertase hydrolysis	80 per cent Ethanol Extraction Acid hydrolysis
1	10.4	6.9	8.6
20	5.1	2.1	3.6

Other Carbohydrates

Stem Temp. °C.	Reducing Sugars 1	Reducing Sugars 2	Fructose	Starch
1	9.92	9.81	9.6	9.4
20	5.57	5.63	4.2	3.4



Figs. 6 and 7. Sucrose content in mg. per g. dry weight of different portions of the tomato plant, as shown at the sketch on the left, after 7 per cent sucrose or water had been applied to the two leaves above the collar. Stems were maintained at 1° C. or 20° C. Means of two experiments.

appear at a 2-3X greater concentration in the colder stem. It therefore seems exceedingly unlikely that the higher sucrose content is produced at the expense of other carbohydrates. The differences should probably be ascribed to differential translocation and respiration at the two temperatures.

In order to study the rate of transport of carbohydrate from leaf down through the stem, the technique of steam girdling the lower portion of the stem and noting the time of first accumulation above the girdle, was used. This technique had previously been used in the tomato plant by Bonner (1944) in studying the loci of formation and the movement of certain substances within the plant. Utilizing large numbers of tomato plants, he girdled many with a steam jet, and left others ungirdled as controls. Stem sections 1.5 cm. in length, above and below the girdle, or at equivalent positions on control plants, were analyzed every day over a five day period for thiamine, riboflavin, pyridoxine, reducing sugars, sucrose, non-protein nitrogen, and protein nitrogen. Sucrose on the fifth day was found to be 87.81 times more concentrated above than below the girdle, whereas the ratio of reducing sugar above and below was 4.91 to 1. Other substances accumulated at varying ratios, some hardly at all. The rate of accumulation of certain of these compounds over several days was very interesting. For example, sucrose had accumulated very little the first day, but had almost reached its maximum on the second day. Thiamin, on the other hand, accumulated

markedly the first day, reaching about 75 per cent of its final concentration. Although steam girdling does not interfere with the transpiration stream, since the plants remain perfectly turgid and normal appearing for about a week, there is evidence of eventual injury. Overton (1911) found that leaves of Cyperus became discolored and dried 3 to 10 days after the stem was steamed, the degree depending upon the length of stem so treated. He concluded that the steaming released poisonous substances which were carried to the upper leaves, but also noted that transpiration was only very slightly decreased by the steaming.

To test accumulation with time, twelve plants were placed in the darkroom at 25° C. They were not decapitated. Half of the plants were steamed near the base, and half left intact, these groups being further subdivided by the addition of either 2° C. collars or 25° C. collars. The three plants thus remaining in each group were harvested at three different intervals after the application of 7 per cent sucrose to all of the plants at 12:00. The plants were harvested 1, 5, and 20 hours after application of sucrose, and the 1.5 cm. sections immediately above the girdle dried and analyzed for sucrose. Unfortunately the results showed no accumulation with time or any other correlation which could be interpreted. The entire experiment was repeated, and the results did not agree at all well. Apparently the fact that there was only one plant to each treatment, in conjunction with the inherent variability or carbohydrate

content of individuals, especially when partially starved, rendered the results too inaccurate to draw any conclusions. However, growth measurements were made on the four plants of the second experiment which were not harvested until 20 hours after sucrose application, the growth increment being shown in Table XIV. The inhibition of growth at the lower temperature can not readily be accounted for by a decreased

TABLE XIV

Increase in Growth of Tomato Plants in mm. Over a 20 Hour Dark Period During Which They Were Supplied Sucrose. Treatment Indicated at Left.

Stem Temp., ° C.	Treatment	Growth, mm./20 hrs.
2	Girdled	6
2	Not Girdled	7
25	Girdled	15
25	Not Girdled	16

carbohydrate translocation at low temperature, because all foliage on the plant (including the two leaves supplied sucrose) was <u>above</u> the cooled stem. The carbohydrate passing from leaves to growing point would not traverse that portion of the stem. A more logical explanation would be that some factor coming from the root and needed for stem elongation, such as caulocaline, is inhibited by the low stem temperature. We do know that cooling causes little or no retardation of the transpiration stream and the inorganic minerals which accompany it. Consequently, it

would be necessary to postulate upward movement of the stem growth substance in the phloem. This, however, would appear unlikely since one would expect a steam girdle to effectively interfere with all transport through the phloem, and yet growth is not significantly different in girdled and non-girdled plants. There is the remote possibility that the growth substance is normally carried upward in the vessels of the xylem, but due to its extreme insolubility at near-freezing temperatures or due to physical changes in the adsorptive properties of the vessel walls at such temperatures, the substance may be almost completely precipitated or crystallized from the transpiration stream.

In order to minimize the inherent differences between individual plants, the next experiment was set up so that six plants received identical treatment. All plants were girdled, and all received 7 per cent sucrose through two leaves. Six had collars maintained at 2° C. and six had 22° C. collars. All plants were harvested together after 48 hours in the dark room, growth being measured at the 24 and 48 hour periods. The sucrose content of 1.5 cm. sections above the girdle of individual plants was determined, the means of which are indicated in Table XV along with growth rates during the dark period. At the same time the plants in the dark room were girdled, two similar plants were girdled in the greenhouse, and allowed to remain there during the same 48 hour period. At the end of this time, sucrose analyses of the sections above the girdle gave

TABLE XV

Sucrose Content of Stem Sections Above the Girdle of Tomato Plants with Leaves in Sucrose and Stems at 2° or 22° C., the Plants having been 48 Hours in the Dark. Growth Rates are also Included. Six Plants per Treatment.

Stem Temp., °C.	Sucrose Concentration (mg./g. dry weight)	Growth in First 24 hrs(mm)	Growth at end of 48 hrs(mm)
2°	2.93 ± 0.729	4.8	12.6 ± 2.13
22°	5.00 ± 0.695	14.8	26.8 ± 2.92

values of 43.6 and 47.9 mg. per g. dry weight respectively, about 10X the value of the plants fed sucrose in the dark. The difference between the means of the sucrose concentrations of plants with stems at 2° and 22° C. is just under significance at the 5 per cent level, attended by 10 degrees of freedom. Difference between the growth resulting from the different temperatures is highly significant. The sucrose values, although not quite significantly different, suggest that over an extended period of 48 hours, greater translocation takes place in the stems held at 22° C. The data on growth would be interpreted essentially the same as in the case of the last experiment.

In addition to the sections just above the girdle, the top 8 cm., the 10 cm. section within the cooling collar, and the section just below the girdle, were all analyzed for sucrose content, at both stem temperatures. The values were all very low, and no significant differences appeared between the two different stem temperature treatments in any case.

The fact that light was many times more effective in

causing the accumulation of sucrose above a girdle than the same sugar applied through the leaves, suggested the use of light as a primary source of assimilates in conjunction with different temperature treatments. A bracket containing four white slimline fluorescent tubes was installed in the dark room. It would give an intensity close to 1000 foot candles when lowered closely over the plants.

The first experiment was again planned so that six plants received identical treatment. All twelve plants were girdled, and then given two photoperiods -- from 12:15 to 22:30 (10 hrs., 15 min.) the first day, and from 8:45 to 21:00 (12 hrs., 15 min.) the second day. During this period six plants had 2° C. stems and six had 24° C. stems (light periods) and 19° stems (dark period). They were harvested immediately after the second photoperiod, at 21:00, each plant being dissected into six sections, as shown in Fig. 8. In addition to the twelve girdled plants, two were illuminated at the same time which had not been girdled. They were dissected in the same manner. Growth, measured over the entire period was not significantly different in any of the treatments, every one of the fourteen plants growing almost exactly 2 mm. during the experimental period. For sucrose analyses, three plants from each group of six were lumped together, thus giving two determinations of each treatment. The two non-girdled plants were also pooled, the values for all plants being indicated in Table XVI. The data unmistakably exhibit the effectiveness of the

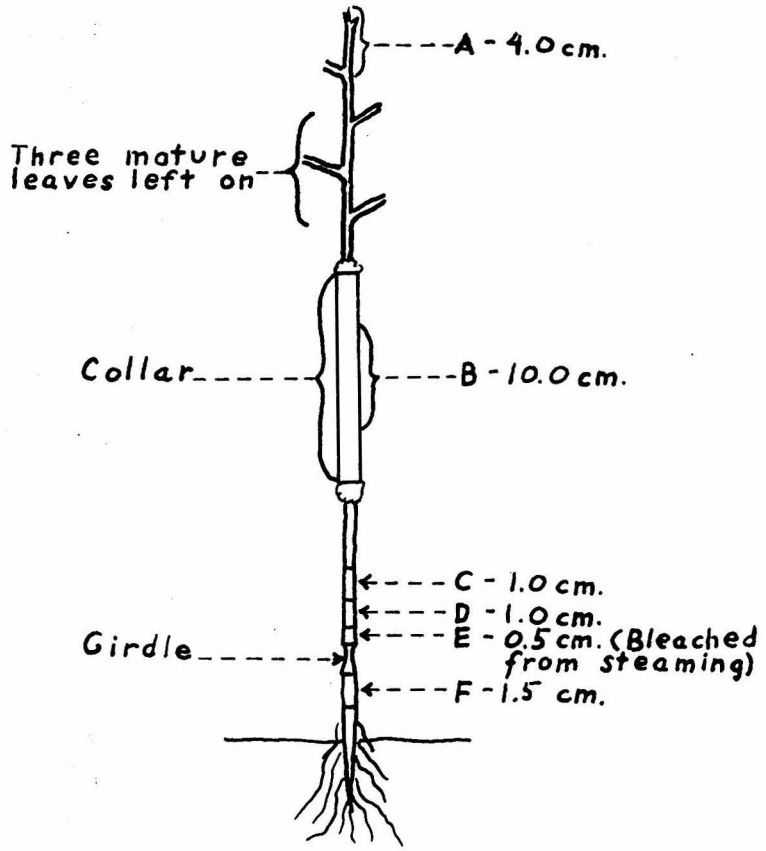


Fig. 8. Various portions of the tomato plant, the sucrose content of which is summarized in Table XVI.

cooled stems in inhibiting the downward translocation of naturally synthesized sucrose over extended periods. Also the apparent complete stoppage of transport by the girdle is clearly demonstrated by the high accumulation of sucrose in the bottom section of non-girdled plants. It was previously noted that accumulation of sucrose above a girdle after only one photoperiod is hardly significantly more when the stem is maintained at 24.5° C. than at 2° C. In conjunction with this phenomenon, the finding of Went (1944a) is of interest. He studied the detailed growth of tomato plants by means of a kymograph, on which growth was

TABLE XVI

Sucrose Content (mg./g. dry weight) of various Parts of Girdled Tomato Plants after two Photoperiod, and stems held at either 2° C. or 19-24° C. Non-girdled Plants with 19-24° C. Stem Included for Comparison. Duplicate Determinations. Sections Correspond to those in Fig. 8.

Treatment	Tops	Center (Within collar)	Upper	Middle	Lower (Just above girdle)	Bottom (Below girdle)
	(A)	(B)	(C)	(D)	(E)	(F)
Girdled, 2° C. Stem	13.5 18.7	4.98 4.85	1.97 2.44	4.10 4.59	3.47 3.49	0.43 0.33
Girdled, 19-24° C. Stem	10.0 10.2	6.74* 21.4*	23.7 34.8	25.9 35.0	26.9 28.6	0.00 0.16
Not Girdled, 19-24° C. Stem	7.0	1.89	24.2	19.4	15.5	21.6

* These widely divergent values suggested an error in analysis, and consequently a second analysis was run on completely new extracts of the same tissues, the values being 5.80 and 21.3 mg./g. respectively. The difference is apparently real.

constantly recorded over extended periods. By use of this method, it was noted that when plants were left in darkness more than 12 hours, growth continued to increase for from 16 to 24 hours, but shortly after that dropped abruptly to zero, due to a depletion of carbohydrates. If plants which had thus come to a standstill in growth were given a ten hour photoperiod, they would fail to grow the following night, but needed a second photoperiod, after which they again resumed their nightly growth. It was thus demonstrated that more than 24 hours was needed for photosynthetic

assimilates formed in the leaves to reach the growing point, at least in sufficient quantity to cause growth.

An interesting corollary has also been found by Went and Carter (1948). Again using the kymograph, it was noted that growth of tomato plants dropped to zero about 33 hours after being placed in darkness. However, growth would again be resumed upon the application of sucrose to the leaves during this dark period. The time interval between such application and resumption of growth appeared to be strongly dependent upon the length of time the plant had been in the dark. For example, if sucrose was given after only 8 hours of darkness, growth was resumed 34 hours thereafter, whereas if given after 42 hours in darkness, growth was resumed in only 9 hours. Such a great difference in rate would seem to indicate either different mechanisms of transport in the two cases, or else that rate is strongly dependent upon concentration gradient, the plants being practically depleted of carbohydrates after 42 hours in the dark, and therefore creating a strong gradient between the sugar-enriched leaves and the growing point.

A study of the long-term effect of a cooled stem upon growth was considered. Four San Jose Canner tomato plants which had been grown in washed river sand and had reached a height of about 90 cm. were selected. Cooling collars 20 cm in length were placed at the center of the plants, about the stems, by removing two leaves. Lower leaves were also removed, thus leaving three mature leaves below

and three young mature leaves above the collar of each plant. Two of the collars were maintained at 7-9° C., and the other two were allowed to remain at room temperature, which was 23° C. day and 17° C. night, the plants being kept in the air conditioned greenhouse for the experiment. Growth was recorded periodically, and on December 22, all of the leaves above the collars of the number 2 plants of each series were removed, in order to study the effect of the cold temperature on transport from the lower leaves upward through the collar to the growing point. Unfortunately the plant so treated, which had the room temperature collar, became infected with fungus about the stem, beneath the collar. Judging from growth rates, the infection must have become toxic just about the time the leaves were removed. Upon completion of the experiment, the other three stems were found to have traces of fungal infection beneath the collars, but no visible damage was discernible. Growth rates are indicated in table XVII. There was a short period of about 8 hours between Dec. 15 and 17th when cold water was not circulating in the collars. The data seem to indicate a very slight retardation of growth in the case of the cooled-stem plants for about the first week, but after two weeks (considering now only the no. 1 plants) the inhibition had seemingly reversed. In the case of the 7-9° plant which had its upper leaves removed, growth continued at a rate equal to about half that of the

TABLE XVII

Growth Rates of Tomato Plants in mm. per Day, between Several Periods of Time, when Centrally Located Collars are Maintained at either 7-9° C. or 17-23° C.

Date of Measurement	7-9° C. Collar		17-23° C. Collar	
	1	2	1	2
Dec. 13, '50				
	13.0	13.5	14.5	13.0
15				
	20.0	18.5	34.0	28.0
17				
	23.5	24.5	25.0	25.5
19				
	19.7	21.3	25.0	23.7
22	(Upper leaves removed this date, no. 2 plants only)			
	25.8	11.4	26.8	0.6 (Fungus)
27				
	28.4	15.0	26.4	0.0 (Fungus)
Jan. 2, '51				
Original Ht.	894	906	876	886
Final Ht.	1365	1230	1390	1693
Total Growth	471	324	514	207

intact plant. This is rather remarkable in view of the fact that the upper leaves, by the time of removal, consisted of considerably more than half of the total leaf area of the plant. Apparently, carbohydrates are readily conducted upward through the cooled stem. It is unfortunate that the figures for the corresponding plant with the 17-23° C. collar are not usable. It would be highly desirable for this experiment to be repeated, utilizing a greater number of plants, and making certain of disinfection before application of the collars.

Another very interesting observation in the above experiment was the considerably larger size and greater greenness of the leaf immediately above, and the one immediately below the collar maintained at the lower temperature, as compared to the same leaves on the plants with room-temperature collars. The latter leaves were, in fact, quite dry and withered by the end of the experiment. Also axillary shoots were observed growing out from the nodes immediately below both of the cooled collars, but not in the case of the room-temperature collars. The explanation of this phenomenon is difficult. It is not unlike the previously described observations of Child and Bellamy (1919, 1920), in which increased outgrowth of buds was noted upon the chilling of stems or petioles of Bryophyllum or Phaseolus. The authors do not attempt to explain the induction of increased plantlets in Bryophyllum leaves with chilled petioles as being due to an inhibition of carbohydrate translocation out of the leaf, but rather interpret it as being due to a stoppage by the cold petiole of bud inhibiting substance formed by the terminal bud and adjacent leaves, and translocated to the leaf in question. This argument might appear logical from one standpoint. Due to the relatively high solubility of most sugars, it would seem impossible for the translocation stream to become saturated with respect to these sugars, even at temperatures just above freezing. On the other hand, many hormones or substances that may inhibit bud growth have an extremely low solubility -- a solubility which in some cases is scarcely higher than

their physiological concentrations, especially under sub-optimal conditions of pH, and the presence of other solutes. The so-called bud inhibiting substance, if it be such a hormone, may not be an exception to this rule. It is conceivable that this substance may reach saturation *in vivo* at temperatures well above freezing, and when cooled to very low temperatures be almost completely crystallized or precipitated from the solution. The fact that chilling of the stems or petioles does not affect the turgor of leaves beyond, and thus apparently does not retard the transpiration stream, is an indication that movement of the inhibiting substance is not through vascular bundles, but is dependent upon physiological activity of the cells -- probably being transported through living protoplasm of the phloem. When the tissue is cooled to a low temperature, this activity is apparently inhibited. However, the inhibition is lost after a few days and the tissue soon becomes acclimated to the new temperature. Indeed, in the bean seedling, temperatures which at first served as a block became completely ineffective after a few days. There is one point that should be noted in the work of Child and Bellamy. In spite of the greater number and development of plantlets on a *Bryophyllum* leaf with chilled petiole, the actual *rate* of development is less rapid in this leaf than in the opposite one which does not have a chilled petiole. Judging from this behavior, one could easily reverse the previous conclusions, or one could even postulate that the low temperature decreases resistance to

outward translocation from the leaf, and that the resultant lowered carbohydrate content causes a slower development of the plantlets. At any rate, the phenomenon is one of great interest. An understanding of its mechanism will probably necessitate investigation from several aspects.

Tracer Studies in Translocation

During the last decade, an increasing amount of experimental work on translocation has been accomplished by the use of radioactive tracers. These investigations have utilized isotopes of phosphorus, nitrogen, carbon, and certain other elements which are required by the plant.

Arnon, et al. (1940) supplied P^{32} to barley and tomato plants in the form of $Na_2HP^{32}O_4$ added to the nutrient. They detected the isotope in the top of tomato plants six feet tall only 40 minutes after addition to the nutrient, the maximum concentration in the nutrient for tomatoes being 28.5 microcuries per liter. Tomato plants were also harvested 36 hours after administration of the isotope, and their leaves and sections of fruit were pressed against non-screen X-ray film for one hour. The resulting radioautographs showed very clearly movement of the isotope into these organs. In order to investigate possible injury due to radiation, barley plants were grown in a nutrient containing 92 microcuries per liter, but no damage was observed even at this high concentration.

Biddulph (1941), also utilizing P^{32} in the same form, studied the diurnal translocation of this isotope from bean leaves. He cut a small flap, which included a vein, from a lower leaf, and dipped it into a solution containing the isotope. It was applied to different plants in this manner at 4 hour intervals, throughout 24 hours, and the activity

of different portions of the plant were analyzed 4 hours after application in each case. Total P^{32} migration from the leaf was thus found to be greatest around 10:00, and least around 22:00. A large percentage moved into the root throughout the day, but ceased with the onset of darkness. However, it again started to move into the roots shortly after midnight, and by 04:00 was almost up to the day rate. This acceleration between midnight and 04:00 is most interesting. Since sunrise was not until a few minutes before 06:00, light was obviously not a factor. Some delicate balance among transpiration, translocation and sugar metabolism must be involved. Movement into the upper stem and leaves was very slight during the morning hours, and stopped entirely after 14:00. Movement appeared to be primarily downward in the phloem and upward in the xylem.

In further work of this type, Biddulph and Markle (1944) found the P^{32} to be localized in single vascular traces, depending upon which vein of the leaf was submerged in the isotope solution. If the fact that the vascular trace from the petiole anastomoses with the vascular cylinder of the stem about one inch below the node is taken into account, then the amounts of P^{32} moving upward and downward from this point are approximately equal. Within the stem, 90 per cent of the P^{32} was found in the phloem, and 10 per cent in the xylem, although the amount in the xylem increased from base to apex due to the slow leaching from phloem to

xylem, followed by rapid upward transport in the transpiration stream. Experiments in which a ring of bark was removed 4 inches below the petiole of the treated leaf showed accumulation of P^{32} above the ring only 1-3 hours after application. Downward movement in the stem was found to be greater than 21 cm. per hour.

P^{32} was also used by Colwell (1942) for translocation studies in Hubbard squash. When a small spot of the isotopic solution was applied to the surface of a leaf, it was not translocated down a petiole which had been scalded, even though the xylem remained intact from the scalding. Movement was thus shown to be in the phloem. If, however, the leaf was almost wilted so that practically no transpiration was taking place, and the tracer then supplied in solution over a large proportion of the leaf, activity could be found beyond the steamed section in only 3 hours, thus indicating a movement through the xylem. Both water and tracer were apparently withdrawn from the leaf surface under these conditions. When a leaf near the shoot tip was treated with radioactive phosphorus, and the plant harvested the next day, movement was found to be predominantly upward into the stem tip. Treatment of a lower leaf in this manner indicated movement to be mostly downward into the roots, thus demonstrating that primary movement was not in the xylem. Finally application to a centrally located leaf resulted in movement to both the tip and the roots. It was also found that when leaves were treated as described above, but sections

of stem analyzed for activity by means of radioautographs rather than by the counter, small amounts of P^{32} arrived near the base of the stem, at a point 200-300 cm. below the treated leaf, in 16 hours. If, however, the leaf was vacuum infiltrated, large concentrations were found throughout the plant in only 3 hours. Such rapid movement was probably through the xylem, since scalding the petiole of the treated leaf failed to stop it.

The recent work of Moore (1949) is of interest. By dividing the roots of maize into two parts, and placing each part in a separate nutrient bath, only one of which contained P^{32}, this author was able to show transport of the isotope to all of the upper parts of the plant within 2 hours. By 6 hours, activity was detected in roots immersed in the other nutrient solution, and at the end of 96 hours the isotope was demonstrated to be in the solution itself. The experiment suggests that downward movement of P^{32} occurs only after it has first been translocated upward to the shoot.

Using $(NH_4)_2SO_4$ labeled with N^{15}, MacVicar and Burris (1948) followed the movement of this isotope when fed to tomato plants in the nutrient. A very rapid increase in concentration of labeled nitrogen to a maximum in about 24 hours occurred in the more mature plant parts, such as the older portion of the stem and roots, and the mature (but not senescent) leaves. Increase of N^{15} concentration in the younger leaves and roots was much slower, taking about 60 hours to reach a maximum. The authors suggest that the

experiment indicates mature tissue as being the site of synthesis of amino acids and other nitrogenous constituents, which are then translocated to more rapidly metabolizing or meristematic portions of the plant, to supplement nitrogenous compounds synthesized there. The rapidity with which the N^{15} first appeared in leaves (less than 2 hours) would indicate with certainty that this initial transport is via the xylem vessels. The secondary transport of the newly synthesized amino acids, being much slower, is undoubtedly an active transport through the living phloem. Experiments of the above type in conjunction with chilling and steam girdling of the stems or petioles should offer interesting results.

Some strange findings were reported by Rabideau and Burr (1945) in which C^{13} in the form of CO_2 was supplied to a centrally located and actively photosynthesizing bean leaf. C^{13} was determined in various parts of the plant after 18-43 hours by means of a mass spectrometer.[1] It was noted that removal of the leaf opposite or removal of the shoot above the treated leaf did not hinder downward translocation of C^{13} into the root. Accumulation in general was by far the greatest in the growing points of both shoot and root, but no transport took place, either upward or downward, past a section of stem scalded with hot wax (100° C.). On the other hand, these workers found P^{32} to pass quite readily through scalded stems. They also found a positive correlation of growth and newly deposited C^{13} in sections

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[1] C^{13} was determined in CO_2 after combustion of the tissue.

of the stem tip, the maximum being about 5 cm. below the stem apex. Under no circumstance was the isotope translocated to the leaf opposite the one fed. Experiments were undertaken in which the receptor leaf was either darkened or in the light, and also where the entire top of the plant above the two leaves was removed, but still no C^{13} was detected in the opposite leaf, even though both sides of the node at the leaf bases contained an equal amount of the isotope. To test this phenomenon from another aspect, the authors starved one leaf by placing it in a black paper envelope for 220 hours. Its poor appearance at the end of this period suggested that it had failed to receive any food from the opposite illuminated leaf, which would corroborate the tracer experiments. These findings do not agree with anatomical studies on the bean plant which show anastomosis of the vessels of opposite leaves, and consequently the phenomenon is strongly indicative of a unilateral translocation in the petiole.

When C^{14} labeled sucrose became available from Dr. Hassid at Berkeley, and later from the Atomic Energy Commission, it was decided to follow its movement through young tomato plants, about 25 cm. high, by means of radioautographs. A solution consisting of 0.2 ml. of 7 per cent sucrose was painted on both surfaces of the five leaflets of an upper but mature leaf of the first plant. The second plant was treated in the same manner except that the solution consisted of 1.084 mg. of the labeled sucrose,

and sufficient normal sucrose to bring the concentration up to 7 per cent. (13.1 mg.) The activity in 1.084 mg. of the tracer was 0.41 microcurie (μc.), and 1.18 x 10^5 counts per minute (c/m). The solutions were made up in 0.8 per cent Drene, as a wetting agent, to produce better adhesion to the leaf. Application to the leaves was at 15:00, and after 24 hours in the dark, the individual leaves and the roots were excised, and the stems were cut into several sections to stop all translocation, all parts being fastened to sheets of paper in as nearly their normal position on the plant as possible. They were pressed in a plant press, and immediately dried in a forced draft oven. After exposure to non-screen X-ray film for 6 days, development showed equally good transport, whether just the tracer was applied, or whether extra sucrose was also added as a carrier. In future experiments the additional normal sucrose was not used. Activity was clearly discernible throughout all of the plant except the lower leaves. It was particularly concentrated in the roots and growing point of the stem. Testing of individual leaves with the Tracerlab monitor indicated a positive correlation between c/m and density on the film. However, the film was more sensitive in picking up very low concentrations, since the weaker images on the film did not register on the counter, when the window was placed over the corresponding part of the plant. This corroborates the previously described findings of Colwell (1942).

The fact that the above experiment demonstrated movement into the roots within 24 hours, suggested a time study in which the plants would be harvested at various intervals after application of the tracer. By feeding C^{13} tagged CO_2 to illuminated young bean plants, Belkengren (1941) found that about 2 hours were required for the synthesis of carbohydrate and its transport in sufficient quantity to be detected at a distance of 30 cm. from the point of synthesis. Exposure of tagged CO_2 in the dark indicated that no unassimilated CO_2 was transported.

The time experiment utilized seven small tomato plants, about 20 cm. high. A solution containing 0.41 μc. of radioactive sucrose in 0.4 ml. of water was painted on both surfaces of the terminal leaflet of one young but mature leaf of each plant at 10:15. The plants were harvested at intervals of 1, 2, 3, 4, 6, 8, and 10 hours after application, each plant being sectioned and dried upon harvesting. After exposure for 22 days, the film was developed. Prints of the radioautographs for the 1, 2, and 10 hour intervals, along with shadowgraphs of the same plants are shown in Fig. 9. In addition to the plants described above, one plant was exposed to the film during the same interval as a blank. An extremely faint image was noted along the film exposed to the stem, thus indicating a minute amount of natural radioactivity in the untreated plant.

The intensity of this image was so weak, however, that the intensity of radiation producing it was probably of the order of 1/1000 or less of that in the more active regions. According to Kamen (1948), normal potassium contains a small percentage of K^{40} (0.012 per cent). This isotope has a half life of 10^9 years and gives off very weak radiation of the negative β type. Such radiation could well be the cause of the blank image, since a considerable amount of potassium is present in plants. There is also the possibility that the pressure of the stem against the photographic film caused the image, and that the leaves, being flatter, did not create sufficient pressure to form an image. At any rate, the intensity of this blank image was so weak, that it in no way interfered with proper interpretation of the radioautographs. The conclusions reached after examination of all autographs may be summarized as follows:

Fig. 9. Radioautographs and shadowgraphs of tomato plants fed C^{14} through one terminal leaflet. Plants harvested after 1, 2, and 10 hours.

1. At the end of 1 hour there was positive but slight translocation of the C^{14} into the roots, and to the other leaflets of the treated leaf. None appeared in the growing point at 1 hour.

2. At 2 hours there was a fairly large amount in the roots, as well as the growing point.

3. At no time did leaves other than the treated one show activity.

4. Several of the films showed clearly the particular vascular bundle through which the isotope passed.

In order to study the effect of temperature by means of the tracer, the following experiment was set up. Four plants, 25 cm. tall, were placed in the dark at 09:00, and 1° C. collars were applied to two of them, the collars being 10 cm. in length. At 14:00, a total of 0.75 µc. of the tracer was painted on the terminal leaflets of these two plants, and on two with collars at room temperature (20° C.). One plant of each set was harvested after 1 hour, the other two plants being harvested after 3 hours. Treatment was as previously described, the resultant radioautographs being illustrated in Fig. 10. Inspection of the autographs shows the following points:

1. A considerable amount of C^{14} in the stems and roots of all four plants, but somewhat more in the roots of the three hour plants.

2. Activity in the growing point appears to be fairly strong in all plants except the one exposed 1 hour with 20° C. stem.

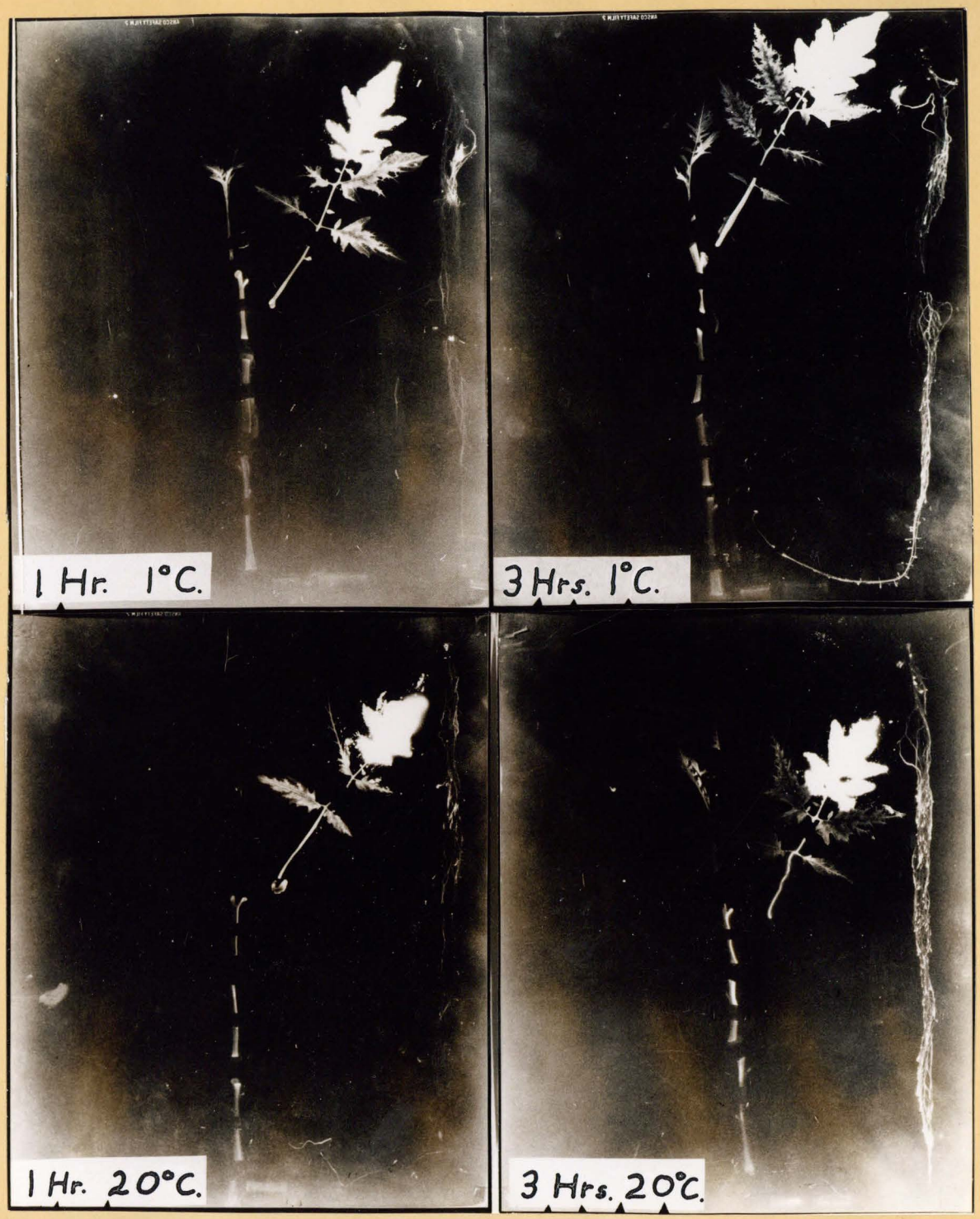

Fig. 10. Radioautographs of tomato plants having had C^{14} applied to the terminal leaflet of one leaf. Plants were harvested after 1 and 3 hours, having had stems maintained at 1° C. or 20° C., as indicated above.

3. There appears to be no difference between translocation (as judged by C^{14} activity in the roots) at the different temperatures, in either the 1 or the 3 hour plants.

4. None of the six mature leaves on each plant showed activity, other than the one to which the tracer was applied. An 80 per cent ethanol extract was made of all leaves which failed to produce an image on the film, and upon concentration onto one counting plate, showed no activity above the background count. This supports the possibility of relatively greater sensitivity of the film, as compared to the counter, in picking up low concentrations of the tracer.

As previously mentioned, it was considered desirable to find in what fractions the C^{14} became incorporated. After a preliminary trial, an extraction was made individually on leaves, stems and roots, as shown in Fig. 11. Only the leaves which showed activity in the radioautographs were used in the extraction, the plants being those described in the last experiment. Although it was not considered ideal to lump together plants exposed both 1 and 3 hours before harvesting, this was done in an effort to conserve all activity possible. To determine the variation in the counter, and what the minimum number of counts above background must be for significance, background was counted for six successive 20-minute periods. The values found, in counts per minute, were: 10.1, 10.4, 9.9, 9.6, 10.0,

All counts shown below are corrected for background, instrumental error, and weight of sample. Low values counted 20 min. or over

All volumes in ml. and wts. in mg.

Dried Tissue

Leaves	0.3367g.
Stems	0.4137g.
Roots	0.2240g.

0.4 ml H_2SO_4
Ether extr'n 25ml, 24 hrs.
Conc. to 7 ml.

Extract → Extr. with 5 ml. 1% $NaHCO_3$ Three times.

Residue → Neutralize Extr. with 80% EtOH, 25 ml. Evap. EtOH Dil. to 20 ml.

Ether Phase → Evap. to small vol. Add 10 ml. ligroin (b.p. 60-70° C.) Extr. with 90% EtOH, 5 ml three times.

Aq. Phase → Acidify to pH 2. Continuous ether extr'n. (2 ml aliquot) 24 hrs., 20 ml ether.

Extract → Ferment with yeast. Incubate in Phos. buffer. Absorb CO_2 in $Ba(OH)_2$

Residue

Ligroin → Pigments, Chlorophylls, Carotenoids, Sterols (A)

EtOH → Xanthophyll Lipids (B)

Ether Phase → Org. & Free Fatty Acids (C)

Aq. Phase → Residue (NaCl, etc.) (D)

$Ba(OH)_2$ Phase → Sugars (E)

Residue → Organic & Amino Acids (F) | Starch, Cellulose, Proteins (G)

	A	B	C	D	E	F	G	H	I
LEAVES									
Vol. extract	7.1	16.7	18.2	18.2	18.2	20.0	20.0	20.0	
Vol. aliquot	1.0	1.0	2.0	1.0	1.5	1.0	1.0	1.0	
Dry wt. aliquot	1.2	1.5	2.5	7.5	10.4	15.7	3.7	10.9	10.0
Wt. fraction	8.5	25.1	22.7	136.4	126.1	314	74	218	336.0
Cts/aliquot	2.9	1.5	26.0	53.2	52.9	33.9	999	711	48.4
Cts/fraction	20.6	25.1	236	968	642	678	19,980	14,200	1598
Cts/mg.	2.4	1.0	10.4	7.1	5.1	2.5	270	65	4.8
STEMS									
Vol. extract	7.2	16.5	17.8	17.8	17.8	1.0	20.0	1.0	
Vol. aliquot	2.0	2.0	2.0	1.0	1.5	1.0	1.0	0.1	
Dry wt. aliquot	2.9	2.1	2.2	8.9	10.6	16.0	4.5	12.1	10.0
Wt. fraction	10.1	17.3	19.6	158.3	125.9	16.0	90.0	121	321.2
Cts/aliquot	0.6	0.2	1.3	1.2	1.0	0.0	13.2	20.0	5.9
Cts/fraction	2.2	1.7	11.6	21.4	11.9	0.0	264	200	189
Cts/mg	0.2	0.1	0.6	0.1	0.1	0.0	2.9	1.7	0.6
ROOTS									
Vol. extract	6.9	16.8	18.7	18.7	18.7	1.0	20.0	1.0	
Vol. aliquot	6.9	5.0	2.0	1.0	1.5	1.0	1.0	1.0	
Dry wt. aliquot	3.0	0.9	0.5	6.6	11.0	13.6	1.9	53.8	10.0
Wt fraction	3.0	3.0	4.7	123.2	137.0	13.6	38.0	53.8	249.8
Cts/aliquot	1.6	0.9	2.1	1.5	0.0	7.3	10.0	142.7	2.8
Cts/fraction	1.6	3.0	19.6	28.0	0.0	7.3	200	142.7	70.0
Cts/mg	0.5	1.0	4.2	0.2	0.0	0.5	5.3	2.7	0.3

Fig. 11

and 10.8. The mean of 10.1 and standard deviation of 0.383 would seemingly indicate that any number of counts roughly 1.2 above background would be highly signficant. However, to be perfectly safe, nothing under 3 counts per minute above background is considered significant. Considering the counts per aliquot (this being the amount concentrated onto one counting plate), we see that only in the leaves does the activity reach the threshold of significance in the case of pigments, including the chlorophylls, carotenoids, and sterols. In none of the organs did activity in the lipids or xanthophylls reach a significant value. Ether extractable organic acids and free fatty acids contained a rather high amount of C^{14}, but only in the leaves. Sugars appear to be in highly significant amounts in the roots and especially in the leaves, but not in the stem. It seems probable that the fermentation on the previous ethanol extract did not go to completion, and that one would therefore expect a larger percentage of activity in the sugars (column F) and a smaller percentage in the aqueous extractable organic acids and amino acids (col. H), than is indicated. The activity of these two fractions would be better considered only as their sum, indicated in col. G. A large amount of activity has accumulated in the cellulose, starch, and protein fraction of the leaves, and a moderate amount in the stems. The rate of conversion of sucrose to other compounds appears to be quite rapid in both the leaves and roots. The activity recovered in the organic

acids is particularly high, as might be expected because of their important role in carbohydrate metabolism. The moderately high activity of the root sugars is of interest, and suggests that some of the first C^{14} in the roots arrives in this form, along with certain of the organic and amino acids.

A determination was also run on the C^{14} activity in the carboxyl carbon of the α-amino acids of the leaves. A 5 ml. aliquot of the sugar-amino acid fraction was incubated in a Thunberg tube with 5 ml. ninhydrin, all buffered to pH 5.0. The upper part of the tube contained 1 ml. of 1/3 saturated $Ba(OH)_2$, the entire tube being incubated 48 hours at 34° C. The $Ba(OH)_2$ and $BaCO_3$ recovered weighed 17.3 mg., and ran 6.3 counts per minute over background. Since the 5 ml. of extract originally contained 4995 c/m, the percentage of C^{14} in the carboxyl group of the amino acids, as compared to total C^{14} in the sugar-acid fraction, was 6.3/4995 or 0.13 per cent. The Thunberg tube was nitrogen filled, and the presence of a moderately large collection of $BaCO_3$ precipitate in the $Ba(OH)_2$ solution, upon completion of the experiment, indicated that a considerable amount of α-amino acids had been decarboxylated, but only an exceedingly small percentage of these contained C^{14}.

Total recovery of C^{14} was only 7.7 per cent, which indicates that a large portion was lost either in respiration or during the processes of drying, mounting, and grinding the tissue.

To investigate the effect of concentration gradient (resulting from previous exposure to light) on rate of translocation, the following experiment was designed. Four 20 cm. plants were placed in the dark room at 17:00, and allowed to remain there the next day, the temperature being 20° C. Four similar plants were left in the greenhouse (day temperature 23° C., night temperature 17° C.) so that they received normal daylight. One plant of each set was steam girdled, and at 16:15 a solution of C^{14} was painted on the terminal leaflet of one young but mature leaf of all plants, both light and dark treated, and the plants were left in the dark. The first two harvests were 15 and 45 minutes after application of the tracer, and included only non-girdled plants, one light and one dark treated plant being taken at each of the harvests. The third harvest, 2 hours after application, included four plants -- both girdled and non-girdled as well as light and dark treated. After treatment in the previously described manner, X-ray films were exposed to the plants for six weeks. Development showed good positive images. The time of exposure can apparently be varied over a moderately wide range, and still provide good density and definition of the image. Colwell (1942) found that exposures involving a time difference of 10X were both completely useful, apparently due to a saturation effect. Such a saturation effect, however, could easily mean that small differences in concentration of the tracer would not be differentiated, particularly if

the exposures were too long. All radioautographs are shown in Fig. 12. along with shadowgraphs of the same plants. There are several points of interest to be noted:

1. Previous exposure to either light or dark apparently makes no difference in the amount of C^{14} transported to the roots, at any given time interval between 15 minutes and 2 hours after application. (The non-girdled dark plant exposed 45 minutes had slightly less activity in the roots than the corresponding light treated plant, but the reverse was true for the 2 hour girdled plants.) Such behavior is suggestive of a depleted carbohydrate content, or a variable concentration gradient, not being of great importance in affecting the rate of this rapid C^{14} migration.

2. Definite activity appears in the roots after only 15 minutes of exposure.

3. Appearance of C^{14} in the growing point and youngest leaves is proportional to the length of time the tracer has been applied, but is not proportional to previous exposure to light or dark.

4. Steam girdles (as shown by arrows on the shadow-graphs) decreased translocation of the tracer, but did not stop it. Considerable activity passed through the girdle, and appears in the roots. A definite accumulation above the girdles is discernible. Lateral movement into a leaf located just above the girdle of the 2 hour dark plant is most pronounced. Movement of

Fig. 12. Radioautographs of tomato plants having had C^{14} applied to the terminal leaflet of one leaf. Plants had previously been exposed to light (L) or darkness (D), and were harvested 15 min., 45 min., and 2 hrs. after application of the radioactive sucrose. Two plants in the 2 hr. harvest were girdled (G), as indicated by arrows on the shadowgraphs, the remaining six plants being non-girdled (NG).

any consequence into leaves other than the treated leaf (except very young leaves) has not previously occurred, this fact being in accordance with the previously mentioned work of Rabideau and Burr (1945). According to the findings of Colwell (1942), a well-watered and turgid squash plant would not transport P^{32} down the xylem (shown by scalding the petiole) when it was applied to the surface of the leaf. The tomato experiment does not corroborate these findings because of obvious transport through the girdle. It is difficult to say whether this phenomenon is simply due to the inefficiency of the girdle, or whether transport was actually through the xylem. All plants were grown in nutrient solution and were maintained on this medium during the experiment, and were consequently completely turgid at all times.

This experiment does not support the conclusion of Tschesnokov and Bazyrina (1930), that translocation is dependent upon the amount of "surplus assimilate" (Assimilate which has not been incorporated into the tissue) in the leaf, and the resulting concentration gradient. It is possible, or course, that the extremely rapid transport made detectable with the tracer, is a completely different type from normal transport.

DISCUSSION

The great complexity of translocation is probably the primary factor brought to light in this and other investigations. Not only do we find possible different mechanisms of transport, variable rates, and variable temperature coefficients for different groups of substances, but these same differences are becoming more apparent even for one group of substances in a single species of plant. Such differences make the acceptance of only one mechanism to explain all transport increasingly difficult. Reasons for the postulation of two mechanisms will be considered later in the discussion.

It would appear that there are at least three distinct rates of sugar translocation in the tomato plant: (1) The tracer work which indicated movement through the greater part of the plant in less than 15 minutes, as seen in the radioautographs, and also the fractionation of different parts of the plant one to three hours after application of the C^{14}, in which activity was recovered in the sugar, starch, and organic acid fractions of the roots. (2) Bleeding experiments which demonstrated rates of 8 to 11 hours. (3) Experiments which involved mass sugar movement as a result of exposure to light or addition of sucrose to the leaves, and subsequent measurement by growth or by tissue analyses, which suggested rates of 24 to 48 hours. That only one mechanism could account for such widely divergent rates of transport of one group of substances in one species of plant seems improbable, if not impos-

ible. The possibility must, of course, be considered that three distinctly different rates of translocation are not being measured in these experiments. For example, it is not impossible that the radioautographs detect the very first trace of material reaching the roots, whereas the bleeding experiments exhibit an increment in exudation rate only after a certain minimum threshold concentration has been achieved in the roots. That bleeding rate is dependent upon the concentration of an activating factor, probably a carbohydrate, within the root itself, appears evident. If such were not the case, it is unlikely that girdling the base of the stem would have any influence on suppressing the sugar effect as it does.

Different rates of transport found by various investigators among <u>different species</u> of plants are probably not necessarily indicative of different mechanisms. Such variations in transport are almost certainly influenced by different rates of synthesis and utilization, as well as certain other factors.

Not only different rates of transport, but also widely different temperature coefficients, as reported in these experiments, strongly suggest the existence of a multi-mechanism type of translocation. The fact that tracer experiments, as well as the bleeding experiments described herein, have not demonstrated a Q_{10} of more than one for translocation is very suggestive of a mechanism of transport unique from the slower more general type movement.

Of the various types of mechanisms proposed for carbohy-

drate translocation, there are several which would appear most likely to account for the data presented in this work. A mass flow type of transport as postulated by Münch (1930), or a modification of this which includes diffusion along plasmodesmata of cross walls and acceleration by protoplasmic streaming within non-vascular tissues in combination with pressure flow through permeable sieve tubes, as proposed by Crafts (1933), would appear to be the most logical explanation for the majority of experiments. The slower movements, involving large amounts of carbohydrate, i.e., transport of low velocity but high capacity, could quite conceivably occur in this manner. Transport of this nature usually involves a time factor of at least 24 hours for movement from leaf to root or leaf to growing point. For example, Table XV, which shows accumulation of sucrose above a girdle only after <u>two</u> photoperiods, demonstrates this slow but voluminous movement. It is not likely that <u>all</u> carbohydrate transport could occur by a high velocity but low capacity type of molecular movement, and in this manner eventually account for the large amounts transported. If such were the case, one would expect an accumulation of carbohydrate above a girdle more or less proportional to time, and such is not the case. At the end of one photoperiod, little or no sucrose is found to accumulate. These findings would corroborate the work of Went (1944b), in which application of a 10 per cent sucrose solution to three leaves of tomato plants, after they had been one day in the dark, was found to cause an increase of 10 to 20 times in

both sucrose and reducing sugars of other leaves, as compared to controls also kept in the dark but not fed sucrose. These great differences became apparent only after 24 to 48 hours after application of the sucrose, and were somewhat dependent upon the length of time the plant had been in the dark.

The question of whether the protoplasmic streaming theory of transport, as postulated by deVries (1885), is an important factor in the experiments herein reported should be considered. To the authors knowledge, the maximum rates of streaming which have been observed are 7.9 mm. per minute in certain cells of aquatic plants maintained for short periods of observation at high temperatures (30-40° C.). Rates observed in phloem parenchyma and companion cells are much slower, the maximum being about 0.4 mm. per minute. The maximum rates reported in the few cases where streaming has been observed in sieve tubes are also of this order of magnitude. It is at once apparent that such rates could not begin to account for the rapid movement detected by tracers. However, that such protoplasmic streaming could be a significant factor in enhancing the slower mass-flow type of transport is certainly not beyond the realm of possibility. If one considers, on the other hand, the <u>average</u> rates of mass transport and the <u>average</u> rates of protoplasmic streaming, it hardly appears possible that streaming could play more than a minor role even in the mass-flow transport.

In searching for a mechanism to satisfy the exceedingly rapid transport demonstrated by the tracer-radioautograph

experiments described in the text, the little-acknowledged hypothesis of Mangham (1917) should not be forgotten. With the knowledge that protoplasm consists of proteins, lipoids, and certain gels, among other substances, which form with water a complex colloidal system, this author postulated that certain of these substances act as adsorbants of sugar, since it had been shown that true adsorption compounds of glucose do exist. In such a colloidal system there would always be a relation between concentration of the solute concerned (sugar in this case) at the surface of the adsorbing phase and within the solvent itself. When a concentration gradient exists, movement of sugar may take place by means of waves of readjustment of equilibrium between these two phases. The rate of movement would approximate that at which condensation on the surface of the adsorbing phase would occur, and should be extremely rapid, particularly when the adsorbing particles were highly concentrated and separated from one another only by a very thin film of solvent.

Another mechanism which may prove successful in explaining the rapid type of transport is that proposed by van den Honert (1932). He was able to demonstrate extremely rapid movement (up to 3 cm. per second) of potassium oleate along an ether-water interface, the interface being along the length of a 100 cm. glass tube. The author suggests that an interfacial boundary between cytoplasm and vacuole may allow for this type of transport in the plant. It seems quite possible that protoplasmic streaming may be a consequence rather than an

agent of such transport. The cause of transport in this case would appear to be nothing more than a concentration gradient of the substance being transported. Inhibition of transport by narcotics agrees rather well with this type of mechanism, since most narcotics are surface active substances and would tend to accumulate at the interface, thus displacing substances which would normally be transported. It is of course questionable whether such a mechanism as this could explain sugar translocation. Although sugars show little or no surface activity at an air-water interface, they have been shown to be positively adsorbed at a coal-water interface, and it is quite possible that the degree of adsorption depends upon the nature of this interface. It may be that the interface between cell wall and cytoplasm, or cytoplasm and vacuole, is of such a nature as to offer positive adsorption toward soluble carbohydrates and other substances. Another alternative is that sugars could be temporarily transformed into some interfacially active form during their course of transport. For example, in van den Honert's experiment, the potassium salt of oleic acid proved to be positively interfacially active, whereas the acid itself was not active. Even if sugars in any form are not positively adsorbed, it is still conceivable that they could be pulled along to a certain extent with natural substances that are so adsorbed. Probably the most serious drawback to such a mechanism of interfacial transport, providing one assumes the cytoplasm-vacuole interface as the path of movement, is the fact that this boundary becomes

rather nebulous in mature sieve tubes. Also, the vacuoles tend to remain as discrete units in the separate sieve tube cells, all of which make it difficult to explain such interfacial movement over any great distance. However, it is possible that young sieve tubes, which have a very sharply defined cytoplasm-vacuole interface, play an important part in translocation, more of which will be said later. Even though they do not transport the major portion of the carbohydrates, they may move small amounts at a very high rate by the above mentioned mechanism.

The rapid movement of C^{14} through the plant, as found in these experiments, would appear most likely to be a molecular movement, not unlike the type observed by Schumacher (1937, 1950) in the case of fluorescein. Such movement was described as being of a molecular nature, independent of the solute and of protoplasmic streaming. Whether such molecular movement is primarily within the cytoplasm, and thus of a particle adsorption nature, or whether it is more of an interfacial type, moving essentially along the plasma membranes, is difficult to say. Earlier work shows conflicting evidence on this point, and the data herein reported do not bear directly on the problem. The possibility of a high velocity but low capacity movement down the xylem can not be completely disregarded. The fact that C^{14} passed a steam girdle would appear to make such a phenomenon not at all impossible, although the more likely explanation of the girdle not being completely effective in stopping transport down the phloem would seem more plausible.

The question of which transport mechanisms are best adapted to account for the experimental data herein reported, as far as the Q_{10} is concerned, is of interest. Q_{10}'s of varying magnitude, but of more than one, would normally be expected of practically all transport mechanisms which have been postulated. However, certain of the experiments described in this work have indicated a transport which is independent of temperature (tracer experiments, and those involving accumulation of sucrose above a girdle after only one photoperiod), or which may even possess a Q_{10} of less than one (bleeding experiments of Went and Hull, 1949). It is suggested that these rapid movements which are almost independent of temperature, or which show a Q_{10} of less than one, are of a molecular nature.

Whether or not a mass flow or modified type of mass flow mechanism could account for movement from leaf to roots in less than 15 minutes, as shown for the C^{14} transport, is certainly open to question. With the knowledge that actively respiring, living cells are necessary for complete efficiency of carbohydrate transport, and that the rate of movement of a liquid through a capillary is inversely porportional to its viscosity, one would normally expect a mass flow type of mechanism to have a Q_{10} of more than one. However, there is a way in which mass flow may be accompanied by a Q_{10} of less than one, as described by Went and Hull (1949). Assuming that carbohydrate transport occurs within the protoplasm of the sieve tube, this protoplasm would offer a certain resistance to movement of

a sugar solution through its interstices, the resistance being proportional to the degree of swelling of the protoplasmic colloids. Since mature sieve tubes have moderately rigid walls, and are almost completely filled with protoplasm, a swelling of the colloids brought about by increased temperature could only result in a decreased cross-sectional area of the protoplasmic solution, with a resultant increment in resistance to mass flow.

In conjunction with this suggestion, there is experimental evidence of permeability being increased by low temperature treatment, as shown by Boon-Long (1941) in the case of cabbage plants held seven days at 5° C. Membranes of the longitudinal sections of petioles were found to be about twice as permeable to water as membranes of the tender unhardened ones, as told by the rate of deplasmolysis. The hardened plants were also found to transpire 2-4 X more than unhardened plants, even though the freezing point depression indicated an osmotic concentration more than twice that of unhardened plants, which, in itself, would tend to decrease transpiration. This would indicate that the increased permeability more than offset the effect of high osmotic concentration on the transpiration rate of the hardened plants. Although the mechanism described by Went and Hull would refer to the permeability of the protoplasm itself, whereas the above experiment indicates an altered permeability primarily of plasmalemma and cell wall, the analogy is of interest, as is the fact that in both cases permeability is increased at low temperature.

A phenomenon not unrelated to this altered permeability is demonstrated by the long term growth experiment, with and without a cooled stem. An inhibition in growth was noted several days after application of the chilled collar, but the treated plants soon overcame the effect of chilling, and grew as well as the controls. The plants apparently became completely acclimated to the low temperature. It is worthy of note that in practically all experiments involving temperature effect on translocation, the investigators have applied local chilling to plants which have been grown throughout their life under moderately warm conditions. This chilling is usually applied only for hours, or at the most for several days, and as such, does not give the plants an opportunity to become acclimated. In many of the long term growth experiments in which certain plants are found to do better at a relatively low temperature, particularly a low night temperature, it is apparent that the plants have become well acclimated, having grown under these conditions all of their life. Consequently, they are able to transport carbohydrates with a high degree of efficiency at low temperatures. Such temperatures are usually considerably below the optimum temperature for the growth process itself. The degree of importance of acclimation is well demonstrated by the experiments of Child and Bellamy (1919, 1920), which are described in the text.

The relationship of sucrose accumulation in various parts of the tomato plant (No. 1 experiment, Table XII) after one day in the dark room, and growth of the entire plant (Table

XIV and XV) during about the same length of time in the dark room, is interesting. When the stem is allowed to remain at room temperature, and sucrose is applied to the leaves, there is a strong tendency for this sugar to accumulate at the top of the collar. On the other hand, a high concentration is found within the stem when it is cooled to 1-2° C., particularly when sucrose is supplied to the leaves. This, along with the fact that various other carbohydrates also occur at a higher concentration in the cooled stems of plants having been supplied sucrose (Table XIII), gives strong evidence of an increased carbohydrate translocation at the lower temperature. The possibility of the high sucrose values resulting from interconversion of other carbohydrates is eliminated by the fact that all carbohydrates appear at substantially higher concentrations within the cooled stem. It appears very likely that these higher values are a result of sucrose application to the leaves, its increased translocation at the low temperature, and partial conversion to other carbohydrates. As far as growth during the dark period is concerned (Table XIV and XV), it appears to be about twice as great when the stems are allowed to remain at room temperature of 25° C. as compared to 2° C. It is difficult to ascribe the lessened growth to decreased carbohydrate translocation, since all leaves on the plant, including those supplied sucrose, were above the cooled stem, and consequently carbohydrate passing from the leaves to the growing point would not pass through the cooled portion of stem. It is more probable that some growth factor arising from

the root, such as caulocaline, was suppressed in upward translocation by the cold stem.

With particular reference to the bleeding experiments reported herein, and those carried out by Went and Hull (1949), in which recording of exudation rate was made by the number of drops per hour falling on a moving paper strip, it has been suggested that evaporation from the drops, as they form on the nozzle tip, may be a source of error at low bleeding rates. To check this possibility, drops of dye were dispensed at various but constant rates by means of a hypodermic syringe, the piston of which was driven with a gear track connected to an electric clock motor. With a knowledge of the syringe capacity (1.00 ml. or 25 drops), and the number of drops falling on the moving paper strip over a given period of time, it is possible to calculate the evaporation in drops per hour. Four experiments were run in this manner, under different conditions of relative humidity and rates of dispension. Recording of the dispensed dye was made both by drops per hour and by weight. In all cases, the circulating fan was left on during the recording, as had been done in the previous experiments.

In the first experiment (room temperature $19.8^{\circ} \pm 0.5^{\circ}$ C., relative humidity 37 per cent), dye was dispensed at a theoretical constant rate of 1.10 drops per hour. The drops actually fell onto the paper at the following rates (in drops per hour): 0.47, 0.50, 0.47, 0.49, 0.53, 0.55, 0.54, 0.59, 0.59, the mean being 0.53. The theoretical rate (1.10) minus

the actual recorded rate (0.53) gives 0.57 drops per hour, the loss due to evaporation.

In the second experiment (room temperature 19.7° ± 0.2° C., relative humidity 42 per cent), dye was dispensed at a theoretical constant rate of 4.40 drops per hour. Drops actually fell at the rate of: 3.5, 3.5, 3.5, 3.5, 3.4, 3.4, 3.4, 3.4 drops per hour, the mean being 3.45. The amount lost due to evaporation was then 4.40 - 3.45 = 0.95 drops per hour.

The third and fourth experiments were modified in that the drops were allowed to fall into a narrow-necked bottle rather than onto the paper strip. The amount dispensed from the syringe minus the amount collected in the bottle, after correction for evaporation from the bottle, would indicate the loss due to evaporation from the nozzle tip. Volume collecting in the bottle was determined by weight, 1.00 ml., as measured by the syringe, weighing 0.9714 g. Details of the two experiments are given in Table XVIII.

In all experiments, evaporation was slightly greater when the dye was dispensed at the faster rate. This appears strange, since over an extended period of time the mean of the surface presented to the atmosphere by the forming drops should be the same, regardless of their rate of formation. The greater loss from evaporation in the first two experiments was undoubtedly due to the low humidity at that time (37 and 42 per cent, respectively). Considering a mean relative humidity of about 50 per cent, 0.40 drops per hour was chosen as an appropriate value to be added to all bleedometer recordings.

Another correction which appeared desirable to incorporate was the osmotic effect of the sugar solution in decreasing the exudation rate. In all bleeding experiments, when water is applied to the leaves rather than sucrose solution, exudation is greater during the first portion of the experiment. This is apparently due to the lower osmotic pressure of the water and its consequent faster absorption by the leaf, the additional water thus becoming added to the transpiration stream. On the average, 1.0 to 1.5 ml more exudation is recorded by the bleedometer for control plants (with leaves in water) than for sucrose fed plants. An approximate measurement of water absorbed by the leaves, after correction for evaporation from the large test tubes in which they were inserted, showed a difference of about 2.8 ml. between control and sugar-fed plants. (3.6 ml. absorbed from the water controls, and 0.8 ml. from the 7 per cent sucrose treated plants.)

TABLE XVIII

	Expt. No. 3	Expt. No. 4
Room temperature, ° C.	19.5 ± 0.3	19.9 ± 0.4
Relative humidity, per cent.	58	55
Theoretical dispension rate, drops/hr.	1.10	4.40
Amount dispensed from syringe, ml.	0.78	0.80
Time of run, hours.	17.50	4.50
Increment in bottle weight, g.	0.5072	0.7021
Loss from bottle due to evaporation, g.	0.0350	0.0090
Increment in wt., corrected for evap., g.	0.5422	0.7111
Corrected increment, in ml.	0.56	0.73
Evaporation from nozzle tip, ml.	0.22	0.07
Evaporation per hour, ml.	0.0126	0.0155
Evaporation per hour, drops. (25 dps/ml.)	0.32	0.39

If the difference between the bleeding rates of the sugar-treated plants and the controls is compared just several hours prior to the onset of the sugar effect, then the value of this difference may be added to the bleeding rate of the sugar-treated plants throughout the entire bleeding period. Such super imposition of the curves at one locus results in a more readily observed delineation of the sucrose effect. In Fig. 1, if the differences between the bleeding rates of the sucrose fed plants with 1° C. stems and the water fed controls are taken for 18:00 and 19:00, and the mean of these differences computed, we see that it amounts to 0.90 drops per hour. (The figures may be more accurately taken from Table I of Went and Hull, 1949.) The entire Sucrose 1° C. curve may then be raised by this value of 0.90, which causes it to become superimposed on the Water 24° C. curve at 18:00-19:00, just before onset of the sugar effect. If the same mean difference is calculated for the Sucrose 24° C. curve, we see that it may be superimposed on the water curve by raising it 0.73 drops per hour. All three bleeding curves are then raised an additional 0.40 drops per hour to offset the previously discussed evaporational loss from the nozzle tip. If these corrections are all incorporated into the data from which the curves of Fig. 1 were plotted, the graph now appears as shown in Fig. 13. It is apparent that there are no basic alterations. The time of upward inflection of the Sucrose 1° C./Water percentage curve takes place about 7 hours after application of the sucrose to the leaves at 11:55, as compared

to 8 hours in the original graph. The upward trend of the Sucrose 24° C./Water curve is somewhat earlier than in the original graph, occurring about 9 hours after application of the sucrose, as compared to almost 12 hours for the original. The differential between the time of upward trend in the two curves is very apparent in both graphs, although it is about 2 hours in the revised graph, compared to 3-4 hours for the original.

One of the most important factors measured by the bleeding experiments is capacity for transport at the different temperatures. The area bounded by the bleeding rate curves for the water control (Water 24° C.) and the sucrose fed plants of the temperature in question (1° C. or 24° C. in Fig. 13), may be taken for the capacity of transport at that particular temperature. As inspection of the curves readily indicates, capacity at the lower temperature is about twice that at 24° C.

Actually, it may be possible to measure velocity of transport, as well as capacity, by such a bleeding technique. If the initial increase in root activity, as determined by the rise in the percentage curve, is a result of the first material arriving in the roots, then the time of this increase may be taken as the time of arrival in the roots. However, the possibility must be considered that the initial rise of the percentage curve is not a measure of the first traces of material reaching the root, but rather the measure of a concentration in excess of a certain threshold.

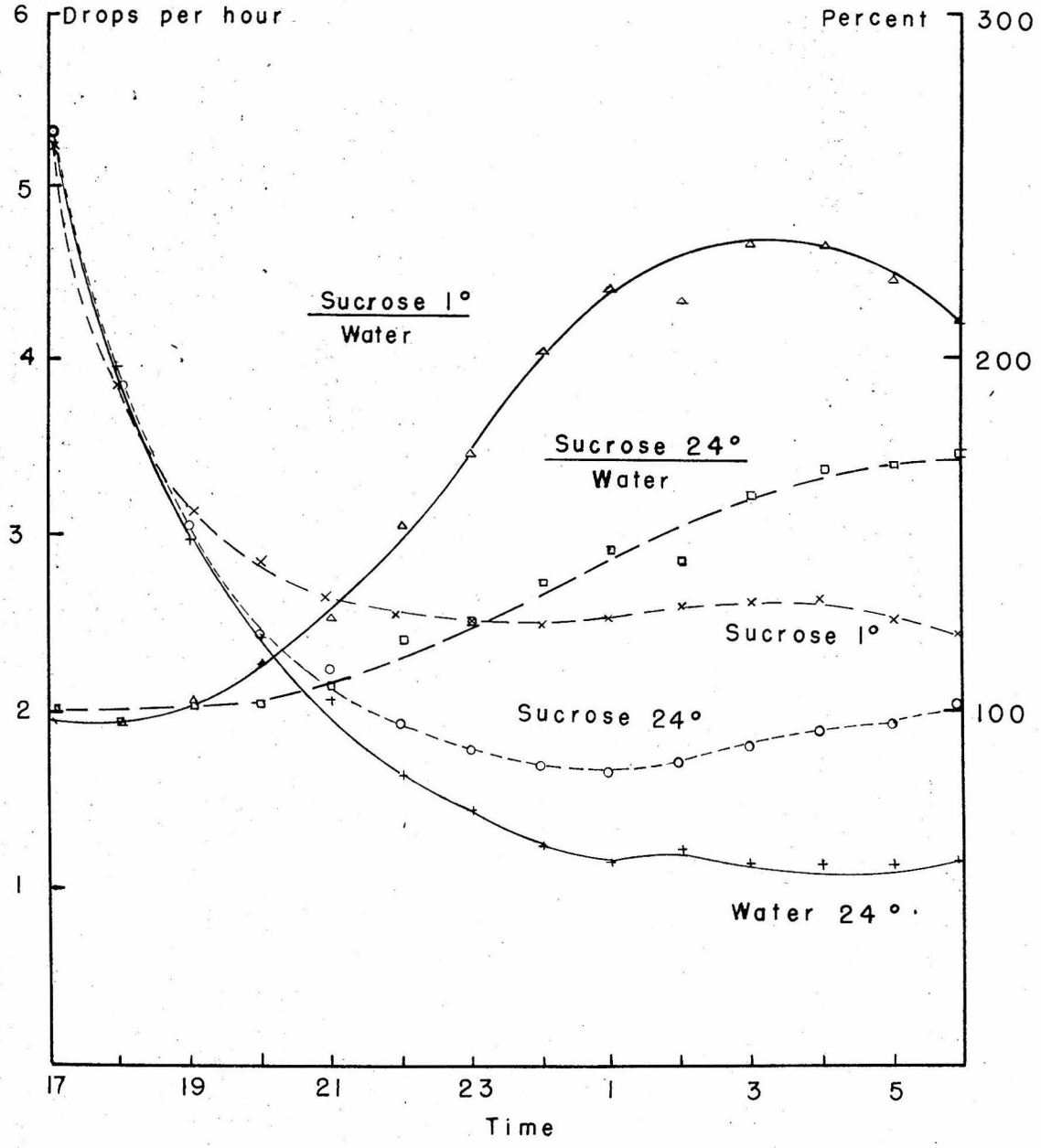

Fig. 13. Revision of graph shown in Fig. 1, with corrections for evaporational loss from the nozzle tip of the bleedometer, and osmotic effect of the 7 per cent sucrose in decreasing exudation.

Although it has been shown by several investigators that general transport of carbohydrates is dependent upon concentration gradient, it appears probable that the rapid molecular type of transport reported in this work acts almost independently of concentration gradient. This is demonstrated first of all by the bleeding experiments, in which the 8-11 hour lag period between sucrose application to leaves and initiation of excess bleeding appears to be essentially independent of previous exposure to light, providing that the plants have not been deprived of light for too long a period prior to the experiment. Secondly, it is suggested by the tracer experiment, the radio-autographs of which are shown in Fig. 12. Here, the rapid transport to the roots under the different conditions of treatment is of about the same magnitude, regardless of whether the plants had been exposed to light immediately prior to the experiment or not.

Also, treatment of the plant during the bleeding experiment appears to have no profound effect on composition of the exudate, as far as application of sugar to the leaves or cooling of the stems is concerned. Apparently none of the root carbohydrates, either naturally occurring or those transported down through application to the leaves, are released to the transpiration stream in any significant amount.

The often-mentioned dependency of translocation on living cells is probably upheld by the majority of these experiments. For example, the fact that application of cyanide to the stem effectively abolished the increased bleeding which normally

results 8-11 hours after sucrose application to the leaves is indicative of this dependency. Numerous other investigators (Wortman, 1890; Czapek, 1897; Curtis, 1928, 1929; Kruseman, 1931; Mason and Phillis, 1936; Dijkstra, 1937) have demonstrated that narcosis or anaerobiosis of the stem or petiole usually results in a reduced transport of organic solutes. Such experiments, when carried on over an extended period of time, until the treated cells are completely inactive, are quite useful. The reduction of transport obtained in such cases may be interpreted as indicating two mechanisms of translocation, one dependent on living cells which are actively respiring, and one operative in inactive tissue. The percentage reduction in transport would determine the relative effectiveness of each system. However, the fact that C^{14} traversed a steam girdle, as shown in Fig. 12, would certainly indicate that not all transport is motivated by living cells. (Assuming that such transport through the girdle did not occur in the xylem, as previously mentioned.)

In studying translocation by means of application of the substance in question to a leaf surface and its determination at some distant locus, it is important to know approximately the amount of time involved for absorption of the substance into the leaf, and finally into the phloem so that it is ready for transport. The experiment summarized in Table V, in which petioles were tightly clamped at various intervals after sucrose application, would roughly indicate that the absorption process takes at least 8 hours. On the other hand, if we consider

carbohydrate transport as occurring by a mass flow mechanism, then pinching the petioles of the only two remaining leaves on the plant might be expected to stop all transport, just as closing the valve in a pipe line would stop the flow of water throughout the entire length of the pipe. If such were the case, it is conceivable that a significant amount of material could have already been transported through the petioles and into the stem by the time the clamps were screwed shut, but that even so, its further transport was thereby hindered. Since mass flow is dependent upon a supply of water which diffuses slowly from xylem to phloem, throughout the leaves and stems, there would undoubtedly be a sufficient amount of such diffusion below the petiole clamps to enable a fair amount of mass flow to continue. A completely correct interpretation of this experiment must therefore be based upon a knowledge of the degree of mass flow possible below such points of isolation, as caused by pinching of petioles.

The controversy of whether carbohydrate translocation involves young or older sieve tubes is one of long standing. In passing, it might be stated that if two mechanisms of transport are postulated, it becomes a possibility that one type of movement tends to take place primarily in young sieve tubes, whereas the other type occurs more in older sieve tubes. Excellent evidence that carbohydrate translocation occurrs essentially in the mature sieve tubes has been offered by Crafts (1938, 1939) and Esau (1935, 1939). However, there are various types of evidence for transport in young sieve

tubes, as described by Clements (1940), James (1933), and Münch (1930). Postulation of an interfacial or molecular type of movement in young sieve tubes which have a definite cytoplasmic-vacuolar boundary and a mass flow type of transport in mature sieve tubes is tempting, but must await further evidence.

It should be stressed that in practically all translocation experiments involving temperature, the different investigators have chilled the petioles of their respective plants, whereas in the present work, as well as that of Went and Hull (1949), the stems were chilled. Although this difference may not at first appear significant, the remote possibility does exist that different mechanisms of transport may be active in the stem and petiole. The very fact that rather marked differences exist in polarity of transport, as shown by Clements (1930), Rabideau and Burr (1945), and Schumacher (1933, 1948), suggests the possibility that certain inherent differences may be present in the conductive elements of the stem and petiole.

In general, it may be stated that the majority of experiments reported herein are in agreement with a mass flow type of translocation. However, certain experiments utilizing the bleeding technique described in the text, and others involving the use of C^{14}, have disclosed a new and extremely rapid type of transport which appears to be almost independent of temperature, or even possesses a Q_{10} of less than one. Such a type of movement is difficult to explain solely on

the basis of mass flow, and it is suggested that this rapid secondary transport is a molecular movement mediated through some type of interfacial or particle adsorption phenomenon.

Upon consideration of the diverse media through which carbohydrate transport takes place, and the many factors which influence its movement, it is not surprising that translocation is one of the most controversial fields of plant physiology. Until the physicist knows more concerning the nature of colloidal systems and adsorption phenomena, and the biologist knows more about the physics of cytoplasm, it is difficult to say <u>exactly</u> how carbohydrate transport takes place within the sieve tube.

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