ammonium chloride (1%) as the sole nitrogen source; it was also able to grow with any of the 19 amino acids present singly[11,13]. The strain of *Sh. flexneri 1a* showed slow growth with ammonium chloride and was able to grow with several amino acids present singly in the medium, the exceptions being serine, proline, tyrosine, and isoleucine. Prompt and luxuriant growth was obtained with *DL*-glutamic acid, 0.01 *M*. The strain of *Sh. dysenteriae 6* was unable to grow in the basal medium with ammonium chloride or single amino acids as the nitrogen source and for its growth a mixture of three amino acids, *DL*-glutamic acid, *DL*-methionine and *DL*-tryptophan (each 0.01 *M*), was required.

The free amino acid pool was analysed after growth in the following media: (a) all three strains on 1.8% nutrient agar (New Zealand)[14]; (b) all three strains in the basal medium containing acid hydrolysed casein (1%) supplemented with *DL*-tryptophan 0.01 *M* and *L*-cystine, 0.0005 *M*; (c) strain 8519 in the basal medium containing ammonium chloride (1%); (d) strain 8516 in the basal medium containing *DL*-glutamic acid, 0.01 *M*; and (e) strain 6342 in the basal medium containing *DL*-glutamic acid, *DL*-methionine, *DL*-tryptophan (each 0.01 *M*) and *L*-cystine, 0.0005 *M*.

After overnight (about 18 h) aerobic growth at 37°C, the cells were harvested and washed once with cold double-distilled water and suspended in double-distilled water to contain between 20–30 mg dry weight of bacteria per ml. The free amino acid pool was present in the supernatant obtained after boiling the cell suspension at 100°C for 10 min[1], cooling the suspension and centrifuging at 3000 r.p.m. The supernatant was evaporated to dryness on a water bath and the residue was dissolved in double-distilled water using one-tenth of the volume of the original supernatant. The amino acids were detected by one-dimensional paper chromatography[4,9]. Known amino acids were used as markers to determine the positions of the fractions[15] and, in recording the results, following MANDELSTAM[9], the fractions were placed within quotations marks.

The composition of the free amino acid pool of the three strains of shigella grown in different media is shown in the Table. MIZUNO et al.[4] found amino acids spots other than glutamic, aspartic and alanine to be negligible in the strains of *Sh. flexneri* and *Sh. sonnei* examined by them. However, in the present study, a number of amino acids were noted in the internal amino acid pool of the shigella strains. The composition of the 'pool' varied with different media in which the cells were grown, but qualitative differences could not be noted in the composition of the 'pool' of the three nutritionally differing strains grown on the same medium. Only slight differences were seen in comparing the 'pool' of the strains grown on their respective minimal media with the 'pool' when grown in casein hydrolysate medium. For strain 8519, with the exception of proline, the 'pool' of the cells from both media was similar. Strain 6342, growing in the basal medium supplemented with four amino acids, showed an additional 'tyrosine' fraction. There were no qualitative differences in the 'pools' of strain 8516 grown in the basal medium supplemented with glutamic acid and grown in casein hydrolysate medium[16].

Composition of the free amino acid pool of *Shigellae* grown in different media (Cells were obtained after overnight growth at 37°C)

Medium of growth	Strain	Amino acid fractions					
		Leucine	Methionine	Tyrosine	Alanine	Glutamic	Aspartic
Nutrient agar	8519	+	+	+	+	+	+
	8516	+	+	+	+	+	+
	6342	+	+	+	+	+	+
Casein hydrolysate	8519	—	+	—	±	+	+
	8516	—	+	—	+	+	+
	6342	—	±	—	+	+	±
Ammonium chloride	8519	—	+	P	+	+	+
Glutamic acid	8516	—	+	—	+	+	±
GMTC	6342	—	+	+	+	+	+

+ Prominent spot in chromatogram; ± Faint spot in chromatogram; — Not seen in chromatogram; P Proline present; GMTC Medium containing glutamic acid, methionine, tryptophan and cystine.

Zusammenfassung. Die Zusammensetzung des «Satzes» (pool) freier Aminosäuren in drei in ihrem Stoffwechsel verschiedene Arten von Shigella, *Sh. flexneri 2a*, *Sh. flexneri 1a* und *Sh. dysenteriae 6*, die in verschiedenen Medien wuchsen, wurde untersucht. Während MIZUNO et al.[4] den Satz von Aminosäuren ausser Glutaminsäure, Asparagin und Alanin in den von ihnen untersuchten Shigellaarten für unwichtig hielten, wurde in unserer Untersuchung in dem Ansatz freier Aminosäuren der Shigellaarten eine Anzahl von anderen Aminosäuren charakteristisch gefunden. Die Zusammensetzung des «Aminosäuresatzes» war bei den verschiedenen Medien, in denen die Zellen wuchsen, unterschiedlich; doch konnten qualitative Unterschiede bei den drei Spezies, die im selben Medium wuchsen, nicht festgestellt werden.

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Department of Bacteriology, University College, Ibadan (Nigeria, West Africa), October 16, 1961.

[13] R. SEN, J. Bacteriol. *80*, 585 (1960).
[14] T. J. MACKIE and J. E. MCCARTNEY, *Handbook of Practical Bacteriology*, 8th Ed. (E. and S. Livingstone Ltd., Edinburgh 1950).
[15] I. SMITH, *Chromatographic Techniques* (William Heinemann Medical Books Ltd., London 1958).
[16] I am grateful to Professor P. COLLARD, Department of Bacteriology, University College, Ibadan, for his helpful advice.

Translocation of Labeled Photosynthate from the Bloom Node Leaf to the Fruit of *Pisum sativum*[1]

Introduction. The specificity of translocation in *Pisum sativum* of phosphorus-32 from the leaf, in whose axil the pod is borne, to that pod has been established. Up to 90% of the phosphorus-32 translocated from the leaf to which the phosphorus-32 is initially applied was found in the pod at the same node[2]. The principal objective of the present study was to learn whether the same specificity exists for carbon compounds as exists for phosphorus.

Materials and Methods. Plants of *Pisum sativum* L. var. Alaska were grown in a controlled environment room having a 75°F light period (12 h) followed by a 65°F

[1] Paper No. 4455, Scientific Journal Series, Minnesota Agricultural Experiment Station.
[2] A. J. LINCK and C. A. SWANSON, Plant and Soil *12*, 57 (1960).

dark period (12 h). Maximum light intensity, at the plants was 1300 f. c. The plants used had pods 12 days past anthesis. The first bloom node leaf of each of four plants was inserted into a glass manifold chamber and sealed with plastic clay. The pressure in the chamber was reduced to 80 mm mercury after which it slowly returned to atmospheric pressure. Radioactive carbon dioxide ($C^{14}O_2$) was generated from $BaC^{14}O_3$ and allowed to enter the sealed manifold chamber. 150 μc of C^{14} was supplied to the chamber. The leaves were allowed to photosynthesize in light at 850 f. c. for 3 h. At the end of this period the contents of the manifold were flushed into a 20% KOH solution.

The plants were divided into six parts: the leaf to which the $C^{14}O_2$ was supplied ('supply leaf'); the stem and leaves above the first bloom node ('stem above'); the stem and leaves below the first bloom node ('stem below'), the roots; the carpel; and the ovules. The plant parts were harvested, frozen and macerated in liquid nitrogen, and the powdered material dried for 24 h at 60°C. The dried plant material was extracted with 80% ethanol and filtered through Whatman no. 1 filter paper. The resulting extracts were brought to a volume of 50 ml, and 2 ml aliquots were removed, taken to dryness in 1-inch planchets, and counted in a proportional counter.

Results and Discussion. Carbon-14 activity in the plant parts is given in Table I. About 90% of the C^{14} activity was localized in the pod (carpel plus ovules). Relatively little (1%) of the C^{14}-labeled compounds was found in the roots and 9% of the transported carbon-14 was found in the stem and the leaves (St_A and St_B). Aliquots of the 80% ethanol extracts from the 'supply leaf', from the carpel, and from the ovules of individual plants were spotted on Whatman no 1 filter paper and subjected to descending chromatography in a *n*-butanol:ethanol (95%): water solvent 52:32:16. Following 40 h of development at a temperature of about 75°F the papers were sprayed with *p*-anisidine-PO_4 and developed at 95°F[3]. Strips ($1^1/_2$ in. wide) from these chromatograms were analyzed with a strip scanner attached to a gas flow Geiger counter with a micromil window (150 mg/cm²). The distance from the origin to the spots developed with *p*-anisidine-PO_4 and the distance from the origin to the zones of radioactivity was measured (Table II). Unlabeled sucrose, fructose, and glucose were tentatively identified in the carpel by their color development and sucrose was found to be located at the same distance (D_2) as the 'known' sucrose from its radioactivity. Radioactive sucrose was also found to be present in the ovules and in the 'supply leaf' following the 3 h period. With the aliquot used, only radioactive sucrose was found in these two plant parts. An aliquot of the extract from the carpel was treated with a 1% solution of invertase, then two-dimensionally cochromatographed with glucose and fructose using *n*-butanol:ethanol (95%):water (52:32:16) and phenol:water (4:1) as the solvent systems.

Only radioactivity in the glucose and fructose 'positions' was found. These results suggest that sucrose is the principal form of sugar accumulated in the leaf and may also be the form in which the photosynthate is translocated to the pod in *Pisum sativum* during the 3 h period. This finding is in agreement with that found for grape[4], and for soybean[5]. The similarity of the distribution pattern for labeled photosynthate and phosphorus-32[2] suggests that the controlling mechanism relating the 'loading' of compounds into the phloem in the leaf and the translocation to sites of utilization in remote organs may be partially similar.

Tab. I. The distribution of 80% ethanol soluble carbon-14 labeled compounds in different parts of *Pisum sativum*

Plant part	CPM × 10^3[a]	$S\bar{x}$%	% C^{14} translocated
Carpel	546	± 28	31 | —90 (pod)
Ovules	1031	± 4	59 |
Stem above	68	± 38	4
Stem below	83	± 38	5
Roots	14	± 26	1
Total	1742	± 4	—
Applied leaf	7879	± 8	—

[a] Each value is the average of the counts made from the respective part of 4 plants.

$$S\bar{x}\% = \frac{S \times 100}{\sqrt{n} \times \text{Avg. CPM}}$$

Tab. II. The identification of sugars in extracts of *Pisum sativum*

Compound	D_1[a]	Leaf D_2	Extract Carpel D_2	Ovules D_2
Sucrose	9.9	9.9	9.3	10.0
Fructose	15.5	0	0	0
Glucose	19.0	0	0	0

[a] D_1 = distance in inches of the spot from the origin developed with *p*-anisidine-PO_4. D_2 = distance in inches of the spot from origin located by measurement of radioactivity.

Zusammenfassung. Es wurde eine starke Verschiebung des C^{14}-Anteils des Achselblattes von *Pisum sativum* zur Frucht hin, die in der Achsel dieses Blattes entspringt, beobachtet. Bis zu 90% des C^{14} (80% Alkoholfraktion) der Pflanze befand sich nach 3 h in der Frucht. Der Hauptanteil des C^{14} dieses Blattes, der Hülse und der Samenanlagen wurde im Rohrzucker festgestellt.

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Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul (USA), August 23, 1961.

[3] S. MUKHERJEE and H. C. SRIVASTAVA, Nature *169*, 330 (1952).
[4] C. A. SWANSON and E. D. H. EL SHISHINY, Plant Physiol. *33*, 33 (1958).
[5] L. P. VERNON and S. ARONOFF, Arch. Biochem. Biophys. *36*, 383 (1952).

Poliovirus Production in HeLa Cells in Presence of Colchicine

Previously the effect of colchicine on poliovirus production was investigated in Maitland cultures of Rhesus kidney[1]). The behaviour of hydrolytic enzymes was also investigated[1], but not the cytological changes. In this preliminary report we describe biological findings on human carcinom a_1 cells (HeLa) treated with large dose of the antimitotic. The cells were subcultured with complete medium[2], in roller tubes, or coverslip cultures.

[1] E. KOVÁCS, Exper. *14*, 295 (1958).