This idea is compatible with the ability of the particles to crystallize, since it has been generally observed that no denatured protein will crystallize.[10]

Preliminary experiments in our laboratory with purified Saukett poliomyelitis virus indicate that it can also be crystallized under conditions successfully applied to the MEF-1 virus. It does not seem unreasonable, therefore, to expect all three types of human poliomyelitis as well as other animal viruses of small size to be capable of crystallization under proper conditions. This crystallization of a virus affecting man and animal emphasizes anew certain basic similarities between animal and plant viruses.

Summary.—The crystallization of human poliomyelitis virus particles from a highly purified and concentrated preparation has been reported for the first time. The appearance of the crystals and the evidence that the crystals are composed of characteristic virus particles, 27 mμ in diameter, are presented and discussed.

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*INFLUENCE OF AUXIN ON CELL-WALL METABOLISM**

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INTRODUCTION

The plant hormone auxin (indole-3-acetic acid, IAA) controls rate of increase in size of the cells of many plant tissues. Thus, excised sections of the oat coleoptile respond to addition of the hormone by increasing more rapidly in length. It is known that the auxin-induced increase in cell volume of the oat section is immediately due to the uptake of water by the cell.[1] Auxin-induced uptake of water by the coleoptile is in turn an osmotic phenomenon.[2] Net water uptake by the coleop-

tile section takes place when the diffusion-pressure deficit of the section, DPD_i, is greater than the diffusion-pressure deficit of the external solution with which the section is in contact, DPD_o. DPD_i is in turn determined by the relation

$$DPD_i = \text{Osmotic concentration of cell contents} - \text{Wall pressure.} \quad (1)$$

It has been indicated[2] that in the oat coleoptile section, auxin may increase DPD_i by decreasing the wall-pressure term of equation (1). This conclusion, arrived at by study of the osmotic relations of the coleoptile, was reached by Heyn in 1931 on the basis of measurements of tissue plasticity as influenced by auxin.[3] The problem of how auxin increases tissue growth rate is then the problem of how auxin brings about relaxation of cell-wall pressure. In the present communication it will be shown that auxin influences a particular facet of the metabolism of the cell wall of the coleoptile. It has been found that the methyl carbon of C^{14}-methyl-labeled methionine is incorporated into cell-wall material. The rate of methyl carbon incorporation into a particular portion of the cell-wall material, the hot-water-soluble portion, is increased in the presence of auxin. Much of the carbon incorporated is removable in a manner characteristic of methyl ester groups. There is also an auxin-induced increase in the rate of incorporation of glucose-derived carbon into the hot-water-soluble fraction. It is suggested that the physical properties of the cell wall may be affected by the hormonally influenced methyl carbon incorporation into and synthesis of particular cell-wall components. The experimental finding is of interest, since it constitutes a metabolic effect of the auxin which is detectable within short time periods.

MATERIALS AND METHODS

The material for the experiments described below consisted of 5-mm. sections cut 3 mm. below the tip of coleoptiles of oat (variety Siegeshafer) seedlings grown in the conventional manner.[4] Primary leaves were removed from all sections. Incorporation of the carbon of varied substances into the cell wall of the section tissue was followed by incubation of sections in the desired C^{14}-labeled substrates, which were obtained from the California Foundation for Biochemical Research. Two hundred sections floating on 4.5 ml. of solution were used for each treatment of each experiment. Solutions were buffered with 0.0025 *M* potassium maleate, pH 4.8; IAA, 5 mg. per liter, was used as the auxin where indicated. At the end of the desired incubation period, the sections were rinsed with water and ground rapidly in a mortar, and the water-soluble and protoplasmic materials were separated from the cell-wall debris by repeated washing and filtration with cold water on a sintered glass funnel. The washed cell-wall material was then separated into constituent groups of components by the procedure outlined in Figure 1, which is adapted from Sinclair and Crandall[5] and Bonner.[6] The names classically applied to the cell-wall fractions of different solubilities are included in Figure 1, but we have no rigorous evidence that the fractions prepared from our material are, in fact, the classical cell-wall materials.

Aliquots of the hot-water-soluble, hot-dilute-acid-soluble, and hot-ammonium-oxalate-soluble extracts of the wall material were counted on copper planchets.

The alkali-soluble fractions were neutralized with 0.5 N and 4 N or 9 N sulfuric acid before plating. The radioactivities of fractions described in Tables 3 and 5

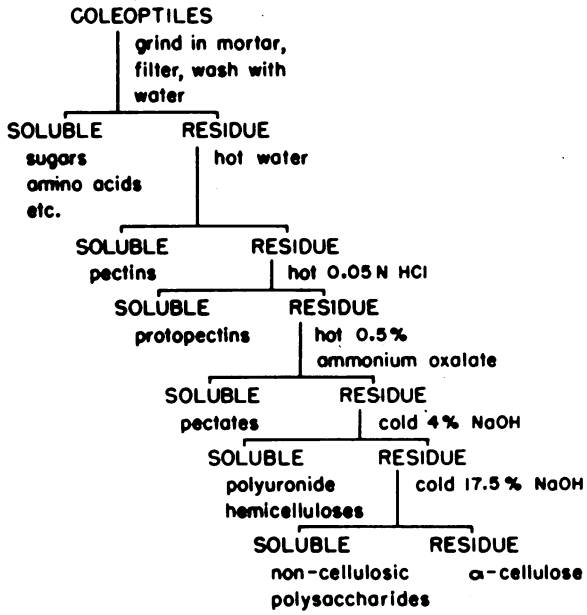

FIG. 1.—Fractionation procedure used for the separation of cell-wall components of oat coleoptile sections.

were counted with a thin-window Geiger-Müller tube. The other experiments were counted in an atmosphere of Q gas with a micromil window tube. All counts are corrected to infinite thinness.

EXPERIMENTAL RESULTS

It has been shown by Sato[7] that the methyl group of methionine provides the methyl ester group of the pectic substances of roots. That the carbon of C^{14}-methyl-labeled methionine is rapidly incorporated into the cell-wall constituents of the oat coleoptile is shown by the data of Table 1. When coleoptile sections are incubated in methyl-labeled methionine, the hot-water-soluble, dilute-acid-soluble, ammonium-oxalate-soluble, and dilute-alkali-soluble fractions of the wall exhibit extensive labeling within 3–4 hours. Other data show that the rate of labeling is approximately constant with time over this period. The cell-wall fractions which are extensively labeled constitute the pectin, protopectin, pectate, and polyuronide fractions of classical cell-wall chemistry.[6] Relatively little labeling is found in the portion soluble only in concentrated alkali or in the alkali-insoluble residue, which includes the noncellulosic polysaccharide and cellulose of the wall. That a portion of the C^{14} incorporated into the wall of the oat coleoptile may be located in methyl ester groups is indicated by the fact that the specific activity of the hot water and dilute-acid-soluble fractions is reduced by 90 per cent or more by hydroly-

sis for 60 minutes in 0.1 *N* NaOH at room temperature, a procedure which de-esterifies the carboxyl group of pectic material.

The data of Table 1 show in addition that the incorporation of the C^{14} of methyl-labeled methionine into the hot-water-soluble and ammonium-oxalate-soluble fractions of the coleoptile cell wall is increased in the presence of IAA. Incorporation into the other cell-wall fractions is but little affected.

That the increased rate of incorporation of methyl carbon into the hot-water-soluble portion of the wall is actually associated with the presence of IAA and is not an artifact associated with the IAA-induced expansion of cell-wall area is indicated by the data of Table 2. For this type of experiment, sections were supplied with

TABLE 1

INCORPORATION OF C^{14} FROM $C^{14}H_3$-METHIONINE INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA (4-HOUR INCUBATION, 0.00133 *M* METHIONINE)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA	CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA
Hot water soluble	732	1,010			
Hot 0.05 *N* HCl soluble	3,490	3,660	17.5 per cent NaOH soluble	81	83
Hot 0.5 per cent ammonium oxalate soluble	118	335	Residue	21	23
4 per cent NaOH soluble	364	261	Total	4,806	5,372

* Initial specific activity of culture solution: −IAA, 74.6 × 10^6 cpm/mM; +IAA, 81.2 × 10^6 cpm/mM.

TABLE 2

INCORPORATION OF C^{14} FROM $C^{14}H_3$-METHIONINE INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA (3-HOUR INCUBATION, 0.3 *M* MANNITOL, 0.00025 *M* METHIONINE)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA	CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA
Hot water soluble	300	551			
Hot 0.05 *N* HCl soluble	2,350	2,175	17.5 per cent NaOH soluble	31	12
Hot 0.5 per cent ammonium oxalate soluble	57	56	Residue	12	19
4 per cent NaOH soluble	366	342	Total	3,116	3,155

* Initial specific activity of culture solution: −IAA, 279 × 10^6 cpm/mM; +IAA, 262 × 10^6 cpm/mM.

C^{14}-methyl-labeled methionine in the presence or absence of IAA but in the presence of a nonabsorbable solute, mannitol, supplied in such a concentration (0.3 *M*) as to just suppress growth of the sections. The data of Table 2 show that in the presence of IAA the rate of incorporation of methyl carbon into the hot-water-soluble fraction of the wall is substantially increased. Incorporation into other fractions of the wall is not influenced by IAA under the conditions of the experiment of Table 2.

It appears that the methyl-derived carbon of the hot-water-soluble and dilute-alkali-soluble fractions of the coleoptile wall is subject to metabolic turnover. This

is indicated by the results of the experiment of Table 3. In this type of experiment, sections were first incubated for 4 hours in methyl-labeled methionine. At the end of this time they were rinsed for 1 hour in water and were then incubated for an additional 17 hours in a high concentration of unlabeled methionine. The exist-

TABLE 3

INCORPORATION OF C^{14} FROM $C^{14}H_3$-METHIONINE INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS (4-HOUR PRETREATMENT, 0.00133 *M* $C^{14}H_3$-METHIONINE, −IAA; 17-HOUR INCUBATION, 0.04 *M* NONRADIOACTIVE METHIONINE, ±IAA)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE ABSORBED/200 SECTIONS: After 4-Hr. Pretreatment	After 17-Hr. Incubation: −IAA	After 17-Hr. Incubation: +IAA
Hot water soluble	952	540	740
Hot 0.05 *N* HCl soluble	6,530	5,950	5,950
Hot 0.5 per cent ammonium oxalate soluble	383	291	453
4 per cent NaOH soluble	1,010	567	1,118
17.5 per cent NaOH soluble	406	187	288
Residue	26	12	22
Total	9,307	7,547	8,571

* Initial specific activities of culture solutions: pretreatment, 64.8 × 10^6 cpm/mM; −IAA, 67.9 × 10^6 cpm/mM; +IAA, 63.5 × 10^6 cpm/mM.

ence of metabolic turnover should be indicated by a decrease in the amount of C^{14} in the wall or in its fractions during the second incubation period. The data of Table 3 show that in the absence of IAA about one-half of the methyl-carbon-derived activity of the hot-water-soluble and dilute-alkali-soluble portions of the wall is lost during the second incubation period. This decorporation of methyl-derived carbon is depressed in the presence of IAA.

The effect of IAA in increasing the rate of methyl carbon incorporation into the hot-water-soluble portion of the cell wall is paralleled at least in part by the effect

TABLE 4

INCORPORATION OF C^{14} FROM GLUCOSE-C^{14} INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA (3-HOUR INCUBATION, 0.3 *M* MANNITOL, 0.00004 *M* GLUCOSE)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL: −IAA	+IAA
Hot water soluble	977	1,093
Hot 0.05 *N* HCl soluble	9,660	9,300
Hot 0.5 per cent ammonium oxalate soluble	69	74
4 per cent NaOH soluble	1,613	1,395
17.5 per cent NaOH soluble	568	375
Residue	428	428
Total	13,315	12,665

* Initial specific activity of culture solution: −IAA, 2,770 × 10^6 cpm/mM; +IAA, 2,470 × 10^6 cpm/mM.

of IAA on the incorporation of the carbon of C^{14}-labeled glucose into the same fraction. The data of Table 4 concern an experiment in which sections were incubated for 3 hours in glucose uniformly labeled with C^{14}, in the presence of 0.3 *M* mannitol. This type of experiment is comparable to that of Table 2, in that the effect of IAA

on incorporation into the cell wall is measured in the absence of complications which might be introduced by effects of growth itself on cell-wall synthesis. The data of Table 4 show that the carbon of C^{14}-labeled glucose is rapidly and extensively incorporated into all the cell-wall fractions. The presence of IAA appears to increase the rate of incorporation into the hot-water-soluble portion. Incorporation into the other fractions of the wall is either depressed or unaffected by the presence of the hormone.

The data of Table 5 concern an experiment in which sections were incubated for 17 hours in an excess of unlabeled glucose after a 3-hour pretreatment in C^{14}-labeled glucose. These data indicate that the glucose-derived carbon of the cell wall, particularly that of the hot-water-soluble and concentrated-alkali-soluble portions,

TABLE 5

INCORPORATION OF C^{14} FROM GLUCOSE-C^{14} INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS (3-HOUR PRETREATMENT, 0.000167 *M* GLUCOSE-C^{14}, −IAA; 17-HOUR INCUBATION, 0.09 *M* NONRADIOACTIVE GLUCOSE ±IAA)*

CELL-WALL FRACTION	CPM/10^5 CPM SUBSTRATE ABSORBED/200 SECTIONS: After 3-Hr. Pretreatment	After 17-Hr. Incubation: −IAA	After 17-Hr. Incubation: +IAA
Hot water soluble	1,077	562	956
Hot 0.05 *N* HCl soluble	14,268	13,901	14,087
Hot 0.5 per cent ammonium oxalate soluble	380	460	352
4 per cent NaOH soluble	4,137	4,754	3,721
17.5 per cent NaOH soluble	1,327	823	1,951
Residue	1,492	1,704	1,751
Total	22,681	22,204	22,818

* Initial specific activities of all culture solutions: 580 × 10^6 cpm/mM.

may be subject to metabolic turnover. This turnover is decreased in the presence of IAA. The bulk of the glucose-derived carbon of the wall does not, however, appear to be subject to turnover. This indicates that the incorporation of glucose carbon represents, in general, net synthesis of cell-wall material, most of which occurs independently of auxin.

SUMMARY

1. The carbon atoms of C^{14}-methyl-labeled methionine and of glucose uniformly labeled with C^{14} are rapidly incorporated into cell-wall constituents of oat coleoptile sections.

2. Incorporation of the carbon of methyl-labeled methionine or of glucose into a particular fraction of the wall, the hot-water-soluble portion, is enhanced in the presence of the plant hormone indole-3-acetic acid (IAA) even if the IAA-induced growth of the tissue is suppressed by a suitable external osmotic concentration.

3. Methyl-derived C^{14} which has been incorporated into the hot-water-soluble or dilute-alkali-soluble portions of the coleoptile wall is slowly lost again when the tissue is incubated in unlabeled methionine. This decorporation of methyl-derived carbon is slowed in the presence of IAA. Similar decorporation of glucose carbon is depressed by IAA.

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† Postdoctoral Fellow, National Science Foundation.

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THE PERMEABILITY OF POTATO TISSUE TO WATER

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Introduction.—Of the possible mechanisms whereby auxin causes plant cells to enlarge, the two which have not been eliminated are, in essence, (*a*) decrease of the mechanical coherence of the cell wall by a metabolic process, resulting in the entry of water under osmotic forces, and (*b*) increased uptake and accumulation of water by a metabolic process, resulting in the mechanical stretching or expansion of the cell wall. In both instances metabolism is also used to redeposit cell-wall constituents and thus to maintain the wall's integrity and thickness. The two alternatives have been described schematically and the evidence for and against them set out.[1, 2] Although there are few critical data distinguishing between the two, the second alternative, often referred to as "nonosmotic water uptake" or a "metabolic water pump," has attracted more attention in recent years. Another explanation sometimes offered, based on an increase of the "permeability" of the cell to water, is closely related to it.

The availability of tritiated water, T_2O or THO, makes possible a fresh approach to this problem, since one can directly follow the entry of radioactive water into a tissue, or its exit therefrom. Water permeability can thus be measured under normal conditions and without subjection to plasmolysis or any other change. The influence of auxin or other factors on the entry can be readily studied.

A somewhat similar approach, using deuterium water, followed by extraction, purification, and determination by densitometric methods has been made by Buffel,[3] by Ketellapper,[4] and recently, using mass-spectrometric methods, by Ordin and Bonner.[5]

Procedure.—The range of the beta particle from tritium is so short that T_2O is detectable only at the extreme surface of a liquid. For this reason the tissue was allowed to take up T_2O beforehand, and the subsequent rate of outflow of the T_2O into H_2O was followed. As material, disks of potato tuber were selected because the rate and duration of water uptake in them has been studied in detail.[6] The