3. In all cases alkylation results in destabilization of the nucleosides or the corresponding moieties in the nucleic acids. At neutral pH, with DNA, 7-alkylguanines and 3-alkyladenines are slowly liberated by hydrolysis, the latter at the greater rate, whereas with RNA slow rearrangements occur, 1-alkyladenine moieties yielding 6-methylaminopurine moieties and 1-alkylcytosines giving the corresponding 1-alkyluracils.

4. The influences of the nature of the 7- and 9-substituents on the rate of hydrolysis or ring-fission of 7,9-disubstituted guanines in the range of pH 5–10 was studied and their pK'_a values were determined.

5. Possible correlations between these reactions and the biological properties of the alkylating agents are discussed.

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Effects of Alkylating Agents on T2 and T4 Bacteriophages

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Loveless (1959a) studied the inactivation of phage T2 by alkylating agents and showed that, with bifunctional agents, inactivation required the reaction of both functional groups. With monofunctional agents inactivation appeared to be due to a dose-dependent and acid-catalysed breakdown of the alkylated phage particles. Studies on the inactivation of tobacco-mosaic virus by alkylating agents (Fraenkel-Conrat, 1961) showed that with

several reagents the introduction of one to three alkyl residues/virus RNA molecule caused 73% inactivation. For both phage and virus it was considered that inactivation resulted from alkylation of the nucleic acid component rather than of the protein.

Brookes & Lawley (1961) have shown that alkylation of nucleic acid occurred at N-7 of guanine moieties, monofunctional agents yielding

7-alkylguanines, and difunctional agents yielding in addition diguanin-7-yl derivatives. The suggestion was made that the difunctional agents might cross-link the twin strands of the DNA molecule and hence prevent strand-separation at the time of DNA replication, and further evidence for the occurrence of such inter-strand cross-links has been obtained (Lawley & Brookes, 1962).

It was thought that bacteriophage of *Escherichia coli* would be a suitable system in which to study in detail the biological effect of alkylation at various extents of reaction and with a number of mono- and di-functional reagents. As in previous studies the use of compounds labelled with ^{14}C and ^3H enabled the extent of reaction of the reagent with the phage constituents to be determined and the nature of the products to be established.

EXPERIMENTAL

Materials. The ^{35}S -labelled mustard gas, i.e. di-(2-chloroethyl) [^{35}S]sulphide (200 $\mu\text{C}/\text{mg.}$), 2-chloroethyl 2-hydroxyethyl [^{35}S]sulphide (200 $\mu\text{C}/\text{mg.}$) and labelled starting materials for other radioactive preparations were obtained from The Radiochemical Centre, Amersham, Bucks.

[^{14}C]Ethyl methanesulphonate (39.5 $\mu\text{C}/\text{mg.}$), [^{14}C]methyl methanesulphonate (42 $\mu\text{C}/\text{mg.}$), [^3H]methyl methanesulphonate (100 $\mu\text{C}/\text{mg.}$) and [^3H]ethyl methanesulphonate (100 $\mu\text{C}/\text{mg.}$) were prepared by reaction of the appropriate labelled alkyl iodide with silver methanesulphonate.

Paper chromatography and radioautography. The methods were as described by Brookes & Lawley (1960).

Radioactivity. DNA was assayed for radioactivity as described by Lawley & Brookes (1963). Labelled proteins were dissolved in hyamine hydroxide (Packard Instrument Co., La Grange, Ill., U.S.A.) and counted in the Packard Tri-Carb liquid-scintillation counter after standing in the dark for 16 hr.

Growth of phage. Phages T2 and T4O^r (osmotic-shock-resistant strain) were used. *E. coli* B was grown with aeration in Roux bottles containing 500 ml. of the minimal salts medium, 'M9' (Adams, 1959) to a cell count of approx. 5×10^8 cells/ml. The culture was infected with approx. 5×10^7 phages/ml. and the aeration increased. When phage T4 was grown tryptophan (10 $\mu\text{g.}/\text{ml.}$) was added to the cell suspension immediately before adding the phage. After about 0.5 hr. the foaming was controlled by smearing a little silicone MS antifoam A (Hopkin and Williams Ltd.) round the top of the air inlet tube. Aeration was continued for about 6 hr. when the cells were lysed by the addition of 30 ml. of chloroform. After standing overnight the suspension was centrifuged at approx. 1000g to remove cell debris and the supernatant, which contained the phage, was again centrifuged in the Spinco model L centrifuge at 22000g. The phage pellet was resuspended by layering on to it 0.15M-sodium acetate or M9 phosphate buffer (Adams, 1959) and allowing it to stand overnight. These processes of low-speed and high-speed ultracentrifuging were then repeated twice more. The final suspension was obtained at approx. 1.5×10^{13} phages/ml. About 50 mg. of phage was obtained from each 500 ml. batch.

Phage assay. Phage was assayed by the agar-layer method (Hershey, Kalmanson & Bronfenbrenner, 1943) with *E. coli* B.

Isolation of deoxyribonucleic acid from phage. From a phage suspension at 1.5×10^{13} phages/ml. a sample of 0.25 ml. was diluted to approx. 2.5 ml. with aq. 6% (w/v) sodium *p*-aminosalicylate, and 2.5 ml. of 90% (w/w) phenol was added. After being shaken for 5 min. the layers were separated by centrifuging and the upper aqueous phase was removed, and the DNA was precipitated by the addition of an equal volume of 2-ethoxyethanol. The DNA was removed on a spatula, redissolved in 2 ml. of 4% (w/v) sodium acetate, again precipitated by ethoxyethanol, and washed in ethoxyethanol, ethanol and finally ether. The yield of dry DNA was approx. 1 mg.

Isolation of protein from phage. The protein of the osmotic-shock-resistant T4O^r phage was precipitated from the phenol layer (obtained in the DNA isolation method) by the addition of 10 vol. of methanol, washed with methanol and ether, and dried.

Phage T2 was incubated in 4M-NaCl at 37° for 10 min. and then rapidly diluted by the addition of 10 vol. of water. The resulting viscous solution was treated at 37° with deoxyribonuclease, and the protein, which remained insoluble, was sedimented, washed by resuspension, sedimented again and dried.

Phage-inactivation by alkylation. In a typical experiment three 1.0 ml. portions of T4O^r phage (1.0×10^{13} phages/ml. in 0.15M-sodium-acetate) were treated respectively at 37° with 30, 60 and 90 $\mu\text{g.}$ of ^{35}S -labelled mustard gas in 0.01 ml. of ether solution. To a control was added a similar volume of ether. After 15 min. 0.01 ml. was taken from the treated samples for phage assay of plaque-forming units by the agar-layer method, and the remainder of these samples used for isolation of DNA. When the methanesulphonates were used reaction was continued for approx. 4 hr. before removing samples for assay. The result of this and similar experiments are shown in Fig. 1.

Phage-inactivation with time, after initial alkylation. In a typical experiment 0.5 ml. of phage suspension (1.5×10^{13} phages/ml. of M9 buffer) was treated at 37° with 1 mg. of [^3H]methyl methanesulphonate in 0.01 ml. of ether and allowed to react for 4 hr. before diluting 0.1 ml. into 10 ml. of M9 buffer. The remaining 0.4 ml. was used for the isolation of DNA and determination of the extent of methylation. The diluted phage was assayed for plaque-forming units immediately and after 24, 72, 96 and 145 hr. Results of this and similar experiments are shown in Figs. 2 and 3.

Degradation of the deoxyribonucleic acid of phage after alkylation. In a typical experiment 0.8 ml. of T2 phage (4.0×10^{12} phages/ml. of 0.15M-sodium acetate) was treated at 37° with 20 mg. of [^{14}C]ethyl methanesulphonate (2.0 $\mu\text{C}/\text{mg.}$). After 4 hr. the suspension was diluted tenfold with 0.25M-sodium acetate, pH 7.2, and incubated at 37°. Samples (2.5 ml.) were taken for DNA isolation after 4, 31 and 85 hr. At 31 and 85 hr. specific radioactivities of the DNA were 88 and 75% respectively of the value at 4 hr.

The results of similar experiments with other reagents are shown in Figs. 4 and 5.

Identification of products from deoxyribonucleic acid of alkylated phage. The methods used were as described by Brookes & Lawley (1960, 1961). Alkylation of either T2 phage or T4 phage with [^{14}C]methyl methanesulphonate,

[^{14}C]ethyl methanesulphonate or 2-chloroethyl 2-hydroxyethyl [^{35}S]sulphide yielded on degradation of the isolated DNA the corresponding 7-alkylguanines, 3-alkyladenines and 1-alkyladenines. ^{35}S -Labelled mustard gas yielded in addition di-(2-guanin-7'-ylethyl) sulphide.

RESULTS

Inactivation of phage by mustard gas was considerable at low extents of reaction (Fig. 1), giving a single-hit inactivation curve, i.e. the plot of log survival against the amount of mustard gas bound approximated to a straight line through the origin. In all experiments alkylation was continued for 15 min. when all added reagent would have either reacted or been hydrolysed and 'second-arm reaction' would be complete. The monofunctional agents, 2-chloroethyl 2-hydroxyethyl sulphide, methyl methanesulphonate and ethyl methanesulphonate all gave inactivation curves of the multi-hit type (Fig. 1), i.e. the plot of log survival against the extent of alkylation showed an upward convexity. The slopes of the curves, however, were very different, being greatest for 2-chloroethyl 2-hydroxyethyl sulphide and least for ethyl methanesulphonate (Fig. 1).

Effects of time of incubation *in vitro* on the inactivation of phage after various extents of alkylation by monofunctional agents showed

Fig. 1. Inactivation of T4O^r phage by alkylating agents. Phage was treated *in vitro* at 37° in either 0.15M-sodium acetate, pH 7.2, or M9 buffer, and assayed for plaque-forming units as described in the text. The extent of alkylation of phage was determined by isolation and radioactive assay of phage DNA; it is expressed on the assumption that the phage DNA contains 7% of phosphate as isolated and has 360000 P atoms/molecule. $\times$, ^{35}S -labelled mustard gas, 30 min. of treatment; O , 2-chloroethyl 2-hydroxyethyl [^{35}S]sulphide, 30 min. of treatment; Δ , [^{14}C]methyl methanesulphonate, 4 hr. of treatment; $\blacksquare$, [^{14}C]ethyl methanesulphonate, 1, 2 or 4 hr. of treatment.

Fig. 2. Effect of time on the survival of phage after alkylation with 2-chloroethyl 2-hydroxyethyl [^{35}S]sulphide. Phage was treated with the alkylating agent for 30 min. at 37° in 0.15M-sodium acetate, then a portion was diluted into 100 vol. of M9 buffer, incubated at 37° and assayed for plaque-forming units after various times. Experimental details are given in the text. O , T2 phage; $\bullet$, T4O^r phage. Numbers on the curves show the number of alkyl groups/phage DNA molecule of mol.wt. 12×10^7 , at the time represented by the first point on the curve.

Fig. 3. Effect of time on the survival of phage after alkylation with [^3H]- or [^{14}C]-methyl methanesulphonate for 4 hr., or [^3H]- or [^{14}C]-ethyl methanesulphonate for 1, 2 or 4 hr., at 37° in 0.15M-sodium acetate, pH 7.2. Portions of the suspensions were diluted after alkylation into 100 vol. of M9 buffer, incubated at 37° and assayed for plaque-forming units after various times. Experimental details are given in the text. Numbers on the curves show the number of alkyl groups/phage DNA molecule of mol.wt. 12×10^7 , at the time represented by the first point on the curve. O , Ethyl methanesulphonate; Δ , methyl methanesulphonate.

Table 1. Yield of alkylated purines obtained by acid hydrolysis of deoxyribonucleic acid from alkylated phage, and ratios of extents of alkylation of protein and deoxyribonucleic acid

Phage was treated at 37° in 0.15M-sodium acetate, the extent of reaction being varied by using different concentrations of reagents, except with methyl methanesulphonate, where samples were withdrawn from the same reaction mixture at various times. DNA was isolated from phage as described in the text and hydrolysed with N-HCl for 5 min. at 100°. The molar ratios of alkylated purines were determined by paper-strip counting of chromatograms of the acid hydrolysate.

Reagent	...	...	Phage T4						Phage T2					
			³⁵ S-labelled mustard gas			2-Chloroethyl 2-hydroxyethyl [³⁵ S]sulphide			[¹⁴ C]Methyl methane-sulphonate			[¹⁴ C]Ethyl methane-sulphonate		
Concn. of reagent (mg./ml.)			0.04	0.09	0.5	0.17	0.5	1.7	3	3	3	10	3.3	
Time of treatment (hr.)			0.5	0.5	0.5	0.5	0.5	0.5	1	2	4	4	22	
Extent of alkylation (moles of alkyl group/mole of DNA)			56	117	510	162	505	1810	1440	2700	3960	610	375	
Relative yields of alkylated purines (percentage of total):														
7-Alkylguanine			61	62	—	—	75	83	81	77	78	92	—	
Di-(2-guanin-7'-ylethyl) sulphide			13	16	—	—	—	—	—	—	—	—	—	
3-Alkyladenine			26	22	—	—	25	17	17	21	20	8	—	
1-Alkyladenine			—	—	—	—	—	—	2	2	2	—	—	
Specific radioactivity of protein/specific radioactivity of DNA			—	1.6	1.6	2.8	2.5	—	1.1	1.1	1.4	—	1.3

Fig. 4. Change with time of the number of alkyl groups/molecule bound to phage DNA after alkylation of T2 phage with 2-chloroethyl 2-hydroxyethyl [³⁵S]sulphide and subsequent incubation of phage in 0.15M-sodium acetate, pH 7.2, at 37°. Experimental details are given in the text.

Fig. 5. Change with time of the number of methyl groups/molecule bound to phage DNA during reaction of T4O' phage with [³H]methyl methanesulphonate and subsequent incubation in M9 buffer, pH 7.1, at 37°. Experimental details are given in the text.

(Figs. 2 and 3) that the decay during the incubation was more marked the greater was the initial alkylation.

With all reagents used the specific radioactivity of the isolated phage protein was of the same order as that of the phage DNA (Table 1). When a phage suspension was alkylated with a radioactive agent the rate of reaction of the reagent with the phage DNA and the subsequent loss of radioactivity from the DNA were similar to those previously found (Lawley & Brookes, 1963) on treatment of isolated DNA (Figs. 4 and 5).

After alkylation of the phage the nature and proportions of the various alkylated bases of the DNA were similar to those found after alkylation of isolated phage DNA (Table 1).

DISCUSSION

It is generally considered, following the work of Herriott (1948), that the inactivation of viruses by alkylation is a result of reaction with the nucleic acid rather than with the protein of the virus. For example, Papirmeister (1961) has shown that those functions of T2 bacteriophage which are dependent

on the integrity of protein components, such as ability to adsorb to, kill and lyse the host bacteria, and ability to inject the phage DNA, are much less sensitive to alkylation by mustard gas than is infectivity. Further, since Hershey & Chase (1952) have shown that only the DNA of the phage participates in phage reproduction, the finding of Loveless (1959*b*) that ethylation of phage *in vitro* could induce mutation again implicated DNA as the site responsible for biological effects of alkylation.

Difunctional alkylating agents are much more effective in causing inactivation of bacteriophages T2 and T4 than are monofunctional agents (Loveless, 1959*a*). This has been confirmed by the present work, which has further enabled quantitative determination of the extent of alkylation of phage DNA required to inactivate. Mustard gas, a typical difunctional alkylating agent, was compared with the corresponding monofunctional agent of equal reactivity, 2-chloroethyl 2-hydroxyethyl sulphide. Inactivation by the difunctional agent was found to be a one-hit process, in agreement with previous studies (Herriott, 1948; Loveless, 1959*a*). The attachment of about 12 molecules of mustard gas to the DNA of a phage particle was required to decrease survival to 37%, indicating that about one-twelfth of the alkylations are lethal.

Far larger extents of reaction of the monofunctional agent were required to produce appreciable inactivation, and the shape of the inactivation curve approximated to that of a multi-hit type (Atwood & Norman, 1949) with an intercept of about 10. This indicates that multiple monofunctional alkylations of a certain type are required within a phage particle for its inactivation. Further, the number of alkylations required to inactivate is greatest when the attached group is ethyl, less with methyl, and least with 2-hydroxyethylthioethyl.

In attempts to investigate the origin of these differences between alkylating agents, the DNA from the alkylated phage was hydrolysed to yield the constituent alkylated bases. As previously found, the sole difference between the products from DNA alkylated with mustard gas and with the corresponding monofunctional agent was the formation by the difunctional agent of di-(2-guanin-7'-ylethyl) sulphide resulting from linkage of two guanine moieties (Brookes & Lawley, 1960, 1961). Since inactivation of phage is thought to be due to alkylation of its DNA it seems likely therefore that the lethal type of reaction resulting from bifunctional alkylation is that involving this cross-linking. Chemical analysis showed that about one out of every six alkylations was of this type, which implies that, of the approximately 12 alkylations/phage particle which are apparently necessary to inactivate, about two would result in cross-linking of guanine moieties. The chemical evidence avail-

able would not exclude the possibility of cross-linking of DNA to protein by the mustard gas, as any bond formed with protein might be hydrolysed by the methods used. The DNA as isolated always contained about 3% of protein which was not increased after alkylation of the phage with either mono- or di-functional reagents. However, the binding of a protein of usual molecular weight would not result in a significant increase in the percentage protein as determined by the methods used. If such cross-linkage of DNA to protein occurred it would be expected to cause inactivation by impairing the ability of the phage to inject the DNA into the host bacteria. However, contrary to this view, Papirmeister (1961) has presented evidence that the ability of phage to inject is much less sensitive to mustard gas than is infectivity. Further, Loveless (1961) has shown that with the bacteriophage ϕ X 174, which contains single-stranded DNA (Sinsheimer, 1959), the large difference in inactivating ability of mono- and difunctional agents is no longer apparent, although some inactivation due to reaction of the second arm was found with di-(2-chloroethyl)methylamine. If cross-linkage of DNA to protein were a significant inactivating reaction it might be expected that this type of reaction with ϕ X 174 would be as easy as, if not easier than, with the 'T even' phages containing double-stranded DNA.

Brookes & Lawley (1961) suggested that the two guanine moieties linked by difunctional alkylation might be derived from opposite chains of the twin-stranded molecule of DNA. Such a reaction would be expected to inhibit the separation of the strands involved in DNA replication according to the scheme of Watson & Crick (1953). Since such a process is probably necessary for phage replication, it follows that such inter-strand cross-linking would result in inactivation of phage at a very low extent of reaction, possibly as low as one cross-link/phage DNA molecule, if it is assumed that all the phage DNA is in one molecule (Rubenstein, Thomas & Hershey, 1961; Cairns, 1961) all of which is required to replicate during phage infection. That inactivation does follow from such low levels of difunctional alkylation has been shown in the present work.

It is clear that the inactivation resulting from monofunctional alkylation follows a different mechanism since a large number of alkylations/phage particle are required to cause significant loss of infectivity (Fig. 1). With methylation and ethylation it is possible that even at the highest levels used in these experiments the act of alkylation itself does not inactivate since the curves (Fig. 3) showing loss of infectivity with time could be extrapolated through the origin. The uncertainty results from the relatively long period of incubation necessary to attain high extents of alkylation owing

to the low rates of methylation and ethylation, during which time decay reactions subsequent to the initial alkylation could have occurred. The effective reaction may therefore be breakdown of the alkylated phage, in agreement with the findings of Loveless (1959*a*). With 2-chloroethyl 2-hydroxyethyl sulphide the alkylation is rapid, and it appears from the influence of time of incubation after alkylation at various levels (Fig. 2) that in this case the initial alkylation itself is probably effective above a certain level of reaction, but less so than the subsequent decay processes. It is notable that with each of the monofunctional agents tested low levels of alkylation failed to inactivate either initially or on subsequent incubation of the alkylated phage.

There is a significant difference in the toxicity towards phage of ethylating and methylating agents and of 2-chloroethyl 2-hydroxyethyl sulphide (Fig. 1). The partition of the bound reagents between DNA and protein showed no significant variations that could explain the differences in toxicity, although possible differences in the sites of reaction within the protein have not been investigated. However, the reasons for excluding protein reaction as discussed above for mustard gas apply here also, and the mutation resulting from ethylation of phage *in vitro* (Loveless, 1959*b*) also implicates reaction with the DNA.

Differences in the mode of reaction of the various reagents with DNA of the phage were therefore studied.

For the various reagents the reactive sites of DNA within the phage were found to be the same as those of isolated phage DNA. In addition the proportions of the various products were independent of the overall extent of reaction and of the nature of the reagent, with the exception that ethylation appeared to produce rather lower proportions of the minor alkylation products (Table 1). This suggested that the differences in toxicity towards the phage might be a result of differences in rates of hydrolysis of the alkylated DNA within the phage. Such differences were found (Figs. 4 and 5) and rates of loss of alkylated bases were similar to those reported for isolated DNA by Lawley & Brookes (1963), being greatest after alkylation by 2-chloroethyl 2-hydroxyethyl sulphide, less after methylation and least after ethylation. The bases hydrolysed from alkylated DNA have been identified as 7-alkylguanines and 3-alkyladenines (Lawley & Brookes, 1963), the latter being the minor products but being hydrolysed at the greater rate at neutral pH. The order of the effectiveness of these agents in causing loss of alkylated purine bases from DNA is the same as the order of their effectiveness as inactivating agents for phage, in agreement with the concept

that inactivation is a consequence of such loss of bases. Since monofunctional alkylation below certain levels does not inactivate, even after allowing time for hydrolyses of the alkylated bases from the DNA (Figs. 2 and 3), it seems that either a considerable amount of damage of this type is necessary or a further process is required which is only significant after loss of such a number of bases. As discussed by Brookes & Lawley (1961), loss of alkylated purines would lead to fission of the sugar-phosphate chain, but breaking of the twin-stranded DNA molecule would be expected only if alkylation on each strand at nearly opposite points had occurred. This would require a considerable extent of alkylation by monofunctional agents as is required for inactivation of phage by such reagents. It is possible therefore that the fission of the double-stranded DNA molecule may be the most effective cause of phage-inactivation. In a study of the inactivation of transforming DNA by heating in aqueous solution, van Sluis & Stuy (1962) have similarly suggested that strand breakage rather than the initial depurination may be the major cause of inactivation.

The observation that only ethylation induces mutation in this system (Loveless, 1959*b*) suggests that mutations of the type so far studied are favoured by retention of the alkylated purine bases, and possibly occur by the mechanism, suggested by Lawley & Brookes (1961), involving incorrect base-pairing between ionized 7-alkylguanines and thymine. Loss of purine bases on the other hand, insofar as it prevents DNA replication, can then be considered as a process potentiating inactivation of phage rather than mutagenesis. It is, however, possible that mutagenesis by another mechanism could result from loss of alkylated purine bases, as discussed by Lawley & Brookes (1963), although so far mutations of this type do not appear to have been detected in phage.

SUMMARY

1. Inactivation of T2 and T4 bacteriophages by isotopically labelled mustard gas, 2-chloroethyl 2-hydroxyethyl sulphide, methyl methanesulphonate and ethyl methanesulphonate was studied, and the dependence on the number of alkyl groups combined with the phage DNA determined.

2. With the difunctional agent a single-hit inactivation curve was obtained, the lethal dose being about 12 alkylations/phage DNA molecule. With monofunctional agents inactivation required a much higher extent of reaction and was a multi-hit process.

3. The sites of DNA alkylated within the phage and their relative reactivities were shown to be identical with those of isolated DNA.

4. The results suggest that inactivation by difunctional agents results from inter-strand cross-linking of DNA. With monofunctional agents it seems likely that the principal mode of inactivation results from degradation of the DNA consequent upon its alkylation.

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Sugar Composition of Oat-Coleoptile Cell Walls

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Much interest centres on the primary cell wall as the probable locus of processes immediately responsible for the growth of the plant cell. However, little has been published about the chemical composition of polysaccharides of primary cell walls of higher plants, especially of growing tissues. Wilson (1961) reported the total monosaccharide composition of growing tobacco-pith cell walls without fractionation. Thornber & Northcote (1961, 1962) fractionated and analysed cell walls of cambium, and of xylem and phloem derived from it, of several species of trees.

Cell walls of the oat coleoptile, a material commonly used for studies on the growth of plant cells, were fractionated by Bishop, Bayley & Setterfield (1958), and analysed partly quantitatively and partly qualitatively. In the present work the monosaccharide composition of the oat-coleoptile cell wall was examined by a different extraction procedure, resembling those that have been employed in most of the radioisotope studies on cell-wall metabolism of this material (Boroughs &

Bonner, 1953; Ordin, 1960, 1962; Ordin, Cleland & Bonner, 1955, 1957).

Results are given on changes in monosaccharides during the growth of oat-coleoptile cylinders, with emphasis on the growth that occurs in the absence of sugar in the medium, when little net increase in cell-wall material takes place (Ray, 1962a). It was thought that under these circumstances it might be possible to detect metabolic alterations of cell-wall polysaccharides that could be involved in the process of cell enlargement, since such changes would not tend to be obscured by the simultaneous addition of new cell-wall material.

METHODS

Plant material. Oats of variety Victory, obtained from Svenska Allmänna Utsädes A.B., Svalöf, Sweden, and of variety Milford, obtained from the National Institute of Agricultural Botany, Cambridge, were grown on moist filter paper, for 1 day under red light followed by 2 days in the dark, at about 26°. Coleoptile cylinders 8 mm. long, begin-