nucleotides. The results of the two-dimensional separation scheme are illustrated in Fig. 1, and the relative distribution of radioactivity is shown in Table I.

Thiouracil thus seems to occupy several of the positions in the nucleic acid of this virus which are normally occupied by uracil. The relatively large proportion oft hiouridine and thiouridine diphosphate liberated by ribonuclease from thiouracil-containing virus nucleic acid is striking. Because of the known specificity of this enzyme, it follows that thiouracil has a tendency to be concentrated at ends of polynucleotide chains, and particularly more at one end than the other.

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On the substrate specificity of glucose oxidase*

Glucose oxidase (notatin) is an enzyme generally considered since the extensive work of KEILIN AND HARTREE[1,2] as highly specific for glucose. The activity relative to glucose with more than fifty related compounds tested was found to be of the order of 1% or less. This high specificity prompted its application to the estimation of glucose in the presence of other sugars[3]. The dependence on a manometric method has acted as some sort of deterrent, which has prevented a wide utilization of glucose oxidase as an analytical tool. The recent adaptation of glucose oxidase activity to colorimetric methods, including its simplification to a paper test[4], will undoubtedly considerably increase the scope of the analytical utilization of glucose oxidase. In attempting to fill in certain gaps of information we have found that 2-deoxyglucose is a fairly good substrate for glucose oxidase. This is important, not only because of the enzymic interest, but also because of the increasing significance of 2-deoxyglucose as an inhibitor of glucose metabolism[5].

For the screening of possible substrates and competitive inhibitors we have utilized the "Tes-Tape" of the Eli Lilly Company, which was kindly made available to us by Dr. J. L. R.-CANDELA. Preliminary tests with low concentrations of glucose indicated that the reciprocal of the time required to obtain a similar degree of color could be used within certain limits as a semiquantitative test of enzyme activity. In some instances the observations were completed manometrically[3], using a preparation of glucose oxidase kindly supplied by the Sigma Chemical Company.

The significant results are summarized in Table I. The relatively high affinity and maximal rate with 2-deoxyglucose obviously prevent the utilization of glucose oxidase for the estimation of glucose in the presence of 2-deoxyglucose. The main reason for other related compounds being poor substrates appears to be a decrease in the affinity of the enzyme for these compounds with respect to glucose. We have observed, both with the Tes-Tape and manometrically, that at 0.05 M concentration the rate with mannose is approximately 1% that with glucose, in agreement with KEILIN AND HARTREE[1]; but the rate of mannose oxidation is essentially proportional to the increase in concentration up to at least 0.5 M. Glucosamine, as could be expected, is not oxidized by the enzyme as the glucosammonium ion, but it is a substrate in the un-ionized form which prevails in alkaline medium. On the other hand, the fact that the apparent activity with maltose can be completely prevented by hexokinase-ATP-Mg clearly indicates that some maltolytic activity is present in the preparation of glucose oxidase. Lack of parallel activity on methyl-α-glucoside makes it easy to understand why the apparent oxidation of maltose was originally interpreted as genuine[1]. The oxidase has no significant affinity for 1,5-sorbitan, although it can

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be considered as 1-deoxyglucopyranose. Fresh solutions of glucono-1,5-lactone in 0.5 *M* citrate buffer, pH 5.5, caused a fairly strong inhibition of the activity with glucose as observed with the Tes-Tape, but no evidence of its significance could be obtained.

It is hoped that these observations, by preventing possible errors in the utilization of glucose oxidase, will contribute to its value as an analytical tool.

TABLE I

SUBSTRATES AND COMPETITIVE INHIBITORS OF GLUCOSE OXIDASE

*Compound**	*Relative rates***	*Michaelis constant**** *M*	*Oxidation coefficient*§
Glucose	100	0.02	1.00
2-Deoxyglucose	25	0.07	0.07
6-Deoxy-6-fluoroglucose	> 3		0.004
Mannose	> 2		0.003
Glucosamine§§	> 2		0.003
Xylose	> 0.4		0.0006
Maltose§§§	—		
N-Acetylglucosamine	—		
1,5-Sorbitan		—	
1,4-Sorbitan		—	

* Obtained as previously described[6,5] except the maltose, which was obtained from the Pfanstiehl Chemical Co. Solutions were allowed to equilibrate before use.

** The unqualified figures are maximal rates. Those preceded by > are the relative rates observed at 0.1 *M* concentration. A dash in this column indicates an undetectable rate at 0.1 *M* concentration (less than 0.05).

*** The last two compounds were tested as possible inhibitors at 0.5 *M* concentration with 0.005 *M* glucose as substrate, using the paper test.

§ Relative rates at substrate concentration (below 0.001 *M*) low enough for essentially first order kinetics even with the substrate of highest affinity. It is equivalent to the phosphorylation coefficient used in the evaluation of substrates for hexokinase[6].

§§ At pH 8.0, using glucose and mannose buffered at this pH as reference standards. No activity was observed with unneutralized glucosamine-HCl solutions, although its pH is within the range optimum for the enzyme.

§§§ In the presence of yeast hexokinase and ATP-Mg in sufficient excess to compete successfully with the glucose oxidase for the glucose liberated by a trace of maltase which is present in the preparations utilized. This contaminating maltase has no appreciable activity on methyl-α-glucoside.

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Ribonucleoprotein from rabbit appendix microsomes*

It is generally believed that microsomes are the main loci of cytoplasmic protein synthesis. Recent findings have shown that they are probably a complex of ribonucleoprotein and protein free of ribonucleic acid (RNA)[1,2,3]. It has also been supposed that it is the ribonucleoprotein moiety that plays the essential part in the synthesis of protein[1,3,4]. In order to analyze the mechanism whereby microsomal ribonucleoprotein participates in the synthesis of specific cytoplasmic

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