with ether. The hydrochloride thus isolated was dissolved in water and neutralized with dilute sodium hydroxide to precipitate the base. Recrystallization from acetone gave 4.5 g. (68% yield) of yellow crystalline product, m.p. 141–142° dec.

Anal. Calcd. for $C_{14}H_{17}N_3O_3$: C, 61.07; H, 6.23. Found: C, 61.12; H, 5.95.

8-(7-Chloro-4-quinolylamino)-6-quinolinol Dihydrochloride (IIIh).—A solution of 2.3 g. (0.01 mole) of 8-amino-6-quinolinol dihydrochloride and 2 g. (0.01 mole) of 4,7-dichloroquinoline in 100 ml. of alcohol was heated at reflux for 15 minutes, whereupon 2.9 g. (71% yield) of reddish yellow crystals precipitated, m.p. 283–285° dec. The product was recrystallized from alcohol, m.p. 295° dec.

Anal. Calcd. for $C_{18}H_{13}ClN_3O\cdot 2HCl\cdot H_2O$: C, 52.25; H, 3.90. Found: C, 52.33; H, 4.12.

8-(7-Chloro-4-quinolylamino)-5-(1-piperidylmethyl)-6-quinolinol Trihydrochloride (IIIi).—A warm solution of 2.6 g. (0.03 mole) of piperidine and 0.3 g. (0.01 mole) of paraformaldehyde in 25 ml. of alcohol was added to 4.1 g. (0.01 mole) of 8-(7-chloro-4-quinolylamino)-6-quinolinol dihydrochloride (IIIh) suspended in 150 ml. of absolute alcohol. The mixture was shaken until complete solution was almost obtained. The trace of insoluble material, considered to be a bis compound similar to VI, was removed by filtration. The filtrate was poured into 40 ml. of saturated alcoholic hydrogen chloride solution. Cooled by ice, the solution gave 5 g. (94% yield) of yellow crystalline product, m.p. 210–212° dec.

Because of the effect of heat in causing the formation of bis compounds, the product was not recrystallized for analysis.

Anal. Calcd. for $C_{24}H_{23}ClN_4O\cdot 3HCl\cdot 3H_2O$: C, 49.48; H, 5.49. Found: C, 49.57; H, 5.73.

LAWRENCE, KANSAS

[CONTRIBUTION FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH]

An Estimation of Pathways of Glucose Catabolism in Yeast[1,2]

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A procedure has been devised for the estimation of the relative extents of participation of the Embden–Meyerhof and shunt pathways in glucose catabolism. It depends on the assumptions: (a) that these pathways are essentially the only ones involved; and (b) that trioses, or 3-carbon compounds derived therefrom, formed *via* the E.M. process arise equally from glucose carbons 1 to 3 and 4 to 6, whereas those derived *via* pentose pathways arise only from carbons 4 to 6. The procedure consists in carrying out simultaneously, with the same tissue or cell preparation, experiments with glucose-1-C^{14} and uniformly labeled glucose. A 3-carbon compound such as lactate or pyruvate, or a 2-carbon compound derived therefrom, *e.g.*, acetate (or acetoacetate), ethanol, etc., is isolated from each, and is purified and assayed for radioactivity. When an appropriate intermediate does not accumulate, one can be "trapped" by the addition of the substance in question. The data from glucose-1-C^{14} provide an indication of the relative incorporation of this carbon in the intermediate, and the data from uniformly labeled glucose provide a correction factor for the endogenous metabolism, whereby "true" values may be calculated for the specific activities of compounds derived from glucose-1-carbon. Under aerobic conditions in *S. cerevisiae* the extent of the shunt process ranged from zero to 30%, and in *T. utilis* it ranged from 30 to 50%. Anaerobically, at least 95% of the glucose was catabolized *via* the Embden–Meyerhof pathway.

It is now recognized that glucose may be dissimilated in certain cells by at least one route which differs from the classical glycolytic pathway of Embden and Meyerhof,[4–6] namely, the hexose monophosphate or oxidative shunt. As presently conceived, this process involves, in successive reactions, the oxidation of glucose-6-phosphate to 6-phosphogluconic acid, decarboxylation of the latter to CO_2 and ribose-5-phosphate, isomerization of the pentose ester to ribulose-5-phosphate, and cleavage of the ketopentose phosphate to triose phosphate and an as yet incompletely identified "diose" closely related to glycolaldehyde.[7] Thus far, most of the evidence for the occurrence of the shunt is based either on the presence of the enzymes involved, or on indirect calculations based on rates of liberation of CO_2 from different positions in the glucose chain. As yet, no direct information is available concerning the relative magnitude of each process in intact, living cells. In the present report a procedure is described, using glucose, variously labeled with C^{14}, which we believe yields a reliable approximation of the extents to which the Embden–Meyerhof (E.M.) and the hexose monophosphate shunt pathways participate in the catabolism of glucose by living cells or tissues. In addition, results are reported of its application to several strains of yeast. Data with regard to other microörganisms and animal tissues will be reported separately.

Basis of Method.—The method is based on the fact that, as shown in Fig. 1, the "symmetrical" cleavage of the glucose carbon chain *via* the Embden–Meyerhof pathway yields two trioses, and ultimately two C2 units, one of which is derived from glucose carbons 1 and 2 and the other from carbons 5 and 6. Thus, any acetate derived from C-1-labeled glucose should have one of four carbons labeled, and its specific activity will therefore be one-fourth that of the labeled glucose-1-carbon, or 1.5 times that of the over-all specific activity of the glucose-1-C^{14}. On the other hand, operation of the shunt should lead to unlabeled acetate, since in this process carbon 1 of glucose is lost by decarboxylation of 6-phosphogluconate. If these two pathways account for essentially all of the glucose catabolized, the specific activity of any acetate produced should fall between 0 and 1.5 times that

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(2) Presented in part at the 124th Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

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(4) F. Dickens, in "The Major Metabolic Fuels," Brookhaven National Laboratory, Upton, N. Y., 1952, p. 134.

(5) B. L. Horecker, *J. Cellular Comp. Physiol.*, **41**, Suppl. 1, 137 (1953).

(6) D. B. M. Scott and S. S. Cohen, *ibid.*, **38**, Suppl. 1, 173 (1951).

(7) F. Dickens, *Biochem. J.*, **32**, 1645 (1938).

of the glucose-1-C^{14}, and the value obtained will be indicative of the proportion of acetate molecules derived by operation of the Embden–Meyerhof process.

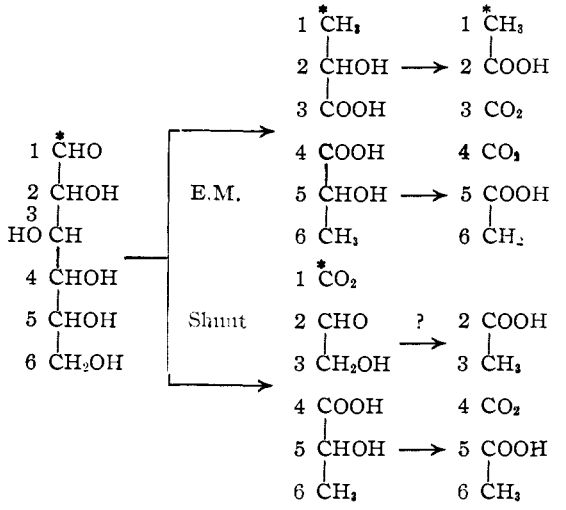

Fig. 1.—Distribution of glucose-1-carbon in 2- and 3-carbon products.

In practice this method is limited by two complications. First, there is in most cells an appreciable endogenous metabolism, yielding carbon which dilutes the exogenous substrate carbon and thus lowers its specific activity to an unpredictable extent. Second, conditions under which acetate or other intermediates accumulate are not always attainable. The first complicating factor may be circumvented by the expedient of carrying out simultaneously, with the same cells under identical conditions, an experiment with uniformly C^{14}-labeled glucose (glucose-U-C^{14}). The difference in specific activity between the uniformly labeled glucose and acetate derived therefrom represents dilution by acetate produced from endogenous carbon, and thus provides a correction factor by which the "true" specific activity of the acetate derived from glucose-1-C^{14} may be calculated. The calculations are made as follows. The relative specific activity (RSA) for the acetates are computed from the formulas

$$\text{RSA} = \text{Sp. act. of acetate times } 100/\text{sp. act. of glucose} \quad (1)$$

$$\text{Corrected RSA} = \frac{\text{Obsd. RSA for acetate from glucose-1-C}^{14}}{\text{Obsd. RSA for acetate from glucose-U-C}^{14}} \quad (2)$$

Assuming that C2 units are produced only by the E. M. or shunt processes, the percentage arising *via* the E. M. process is

$$\text{E.M.} = \text{Corrected RSA times } 100/1.5 \quad (3)$$

The specific activities represent the counts per minute observed using our standard radioactivity assay procedure. Samples in the form of barium carbonate, spread in an "infinitely thick" layer in aluminum planchets, 7.5 sq. cm. in area, are counted using a conventional mica window Geiger tube.

When acetate or another convenient intermediate does not accumulate, a pool of unlabeled acetate may be added to "trap" labeled molecules. Here again, a corrected value for the RSA can be calculated in the same way, on the reasonable assumption that dilution by both endogenous carbon and added carrier carbon is identical for each labeled sugar when experiments are conducted under exactly the same conditions.

In this discussion, acetate is used as an illustrative example. Obviously other 2-carbon intermediates which have an origin common with acetate, *e.g.*, ethanol or acetaldehyde would also serve, as would derivatives of acetate, such as acetoin, acetoacetate, etc. Inasmuch as the method is essentially based on the origin of triose molecules, any substance derived from triose, including lactate or pyruvate, would also be suitable, with slight modifications in the calculations. Thus the procedure is of wide applicability.

Experiments and Results

The organisms used in this investigation were a commercial strain of *Saccharomyces cerevisiae* (ordinary Fleischmann's baker's yeast) and a strain of *Torulopsis utilis* used in our laboratory for biosynthetic studies.[8] Uniformly labeled glucose was obtained from the Nuclear Instrument Corporation of Chicago, and the glucose-1-C^{14} and -6-C^{14} from the National Bureau of Standards through the kindness of H. S. Isbell, on allocation by the Atomic Energy Commission. Aliquots of the cells of approximately 0.5 g. were suspended in 100 ml. either of distilled water or 0.1 *M* *p*H 7.4 phosphate buffer contained in two 250-ml. erlenmeyer flasks; one contained the uniformly labeled and the other the C-1-labeled glucose in amounts of approximately 5 m*M*. The flasks were shaken on a reciprocating or a rotary shaker, while a stream of oxygen was drawn through the flask and into a glass bead tower containing CO_2-free NaOH for absorption of respiratory CO_2. In anaerobic experiments the flasks were swept with a stream of tank nitrogen. Particular care was taken to maintain both flasks under identical conditions. At the end of the experimental period the cells were killed by adding 6 ml. of 18 *N* H_2SO_4, they were centrifuged off, and ethanol and acetate which accumulated were isolated from the supernatant fluid by steam distillation essentially as described in previous studies.[9] The distribution of C^{14} in acetate was determined by the Schmidt degradation as modified by Phares,[10] and glucose by the procedure of Seifter, *et al.*[11]

Since acetate does not ordinarily accumulate in large amounts during the oxidation of glucose by normal yeast cells,[9] in most experiments data were obtained by isolation and assay of ethanol, which was always produced in isolatable quantities. In some instances, acetate was added as a cosubstrate as a "trapping" agent; in others the small amounts of acetate which accumulated were isolated and assayed.

Results of two experiments which are typical of nine experiments thus far conducted are given in Table I. The first experiment was carried out with resting cells of *S. cerevisiae* depleted of endogenous nutrients by vigorous aeration for 16 hours at room temperature. The cells were suspended in distilled water, and unlabeled sodium acetate was added as a cosubstrate.

Essentially all of the glucose was utilized during the 4.5 hours of the experiment, and there was extensive replacement of the added acetate by labeled acetate derived from both the C-1 and the uniformly labeled glucose as indicated by their high RSA. With the respective glucoses incorporation of the labeled carbon was 13 and 10% of the initial activity. The close correspondence in yields of products obtained with glucose-1-C^{14} and glucose-U-C^{14} was regarded as a good indication that our assumption of constancy of conditions was being fulfilled. The acetate derived from glucose-U-C^{14} had an RSA of 30.2% and that from glucose-1-C^{14} had an

(8) M. Strassman and S. Weinhouse, THIS JOURNAL, **74**, 1726 (1952); **74**, 3457 (1952); **75**, 1680 (1953).

(9) S. Weinhouse, R. H. Millington and K. F. Lewis, *ibid.*, **70**, 3680 (1948).

(10) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951).

(11) S. Seifter, S. Dayton, B. Novc and E. Muntwyler, *ibid.*, **25**, 191 (1950).

TABLE I

PRODUCTS OF AEROBIC LABELED GLUCOSE DISSIMILATION BY RESTING, DEPLETED CELLS OF *S. Cerevisiae*

	Experiment 1 — unbuffered, 4.5 hours						Experiment 2 — *p*H 7.4, 4.0 hours					
	Glucose-1-C^{14}			Glucose-U-C^{14}			Glucose-1-C^{14}			Glucose-U-C^{14}		
	m*M*	RSA	Total activity, %	m*M*	RSA	Total activity, %	m*M*	RSA	Total activity, %	m*M*	RSA	Total activity, %
Glucose, start	5.0	100	100	5.0	100	100	5.0	100	100	5.0	100	100
Glucose, end	Nil.	...	...	0.015	...	...	0.48	...	...	0.43	...	...
Acetate, start	5.0	0	0	5.0	0	0	0	0	0	0	0	0
Acetate, end	4.58	44.8	13.4	4.96	30.2	10.0	0.34	...	...	0.24	...	...
Respiratory CO_2	12.4	41.2	17.0	12.3	62.5	25.8	10.9	32.4	11.7	10.8	96.2	34.7
Ethanol	1.49	100.1	10.0	1.25	63.8	5.3	4.45	97.0	28.7	4.96	78.4	26.0

TABLE II

EXTENT OF OCCURRENCE OF EMBDEN–MEYERHOF PROCESS IN AEROBIC GLUCOSE DISSIMILATION BY YEAST

Expt. no.	Time of expt. hr.	Yeast strain	Conditions	Corrected RSA Ethanol	Corrected RSA Acetate	CO_2	Total C_2 units formed *via* E.M. process, %
1	4.5	*S. Cerevisiae*	Depleted, unbuffered, acetate trap	1.57	1.48	0.66	100
2	4.0	*S. Cerevisiae*	Depleted, *p*H 7.4	1.24	..	0.34	83
3	2	*S. Cerevisiae*	Depleted, unbuffered	1.32		0.32	88
4	2	*S. Cerevisiae*	Depleted, *p*H 7.4	1.40	1.30	0.29	90
5	2	*T. utilis*	Fresh, *p*H 7.4, acetate trap	..	1.06	1.00	71
6	2	*T. utilis*	Fresh, unbuffered	1.22	..	0.63	81
7	2	*T. utilis*	Fresh, *p*H 7.4	1.11	1.10	0.69	74
8	2	*T. utilis*	Depleted, *p*H 7.4	..	1.14	0.65	76
9	3	*T. utilis*	Growing cells	0.95	1.03	1.01	66

RSA of 44.8%. Thus the corrected RSA for the acetate derived from glucose-1-C^{14} was 44.8/30.2 = 1.48. The RSA for ethanol derived from glucose-1-C^{14} was 100.1; when corrected by the value of 63.8 observed for alcohol derived from glucose-U-C^{14}, the RSA was 100.1/63.8 = 1.57. Thus, under the particular conditions of this experiment, with depleted cells of *S. cerevisiae*, the corrected RSA's were close to 1.5, the theoretical value for exclusive operation of the Embden-Meyerhof process. The data on respiratory CO_2 in this experiment are also in accord with extensive operation of the E.M. path. If the shunt were the major pathway, glucose-1-carbon would have been preferentially converted to CO_2; this effect would have been particularly striking in this experiment in which relatively large amounts of carbon accumulated as ethanol. However, the higher RSA of CO_2 from glucose-U-C^{14} clearly indicates that glucose carbon 1 was less rapidly oxidized than other glucose carbons. This finding is in harmony with the expectation that in the Embden–Meyerhof process carbons 1 and 6 of glucose, which form the methyl carbons of acetate, would be less rapidly oxidized than carbons 3 and 4, which are lost prior to acetate formation, or carbons 2 and 5 which yield the carboxyls of acetate.

The second experiment of Table I differs from the first in the use of phosphate buffer to keep the *p*H constant throughout the incubation period of 4 hours, and in the omission of acetate as a cosubstrate. In this experiment specific activity comparisons on the ethanol which accumulated also indicate that the Embden–Meyerhof process is the major one. The corrected RSA for ethanol in this experiment was 97.0/78.4 = 1.24, which indicates that 1.24/1.50 = 83% of the ethanol molecules in this experiment arose via the Embden–Meyerhof process. Again, a higher RSA of respiratory CO_2 from glucose-U-C^{14} is in further accord with the major occurrence of the Embden–Meyerhof path. The fact that the corrected RSA was substantially below the theoretical value of 1.5, however, showed that the shunt or some other similar mechanism was also operative. This observation has been consistently repeated in subsequent experiments.

In all, nine experiments of similar type were carried out with *S. cerevisiae* and *T. utilis* under various conditions, and corrected RSA values of the C_2 compounds are summarized in Table II. In four of these, both acetate and ethanol were isolated. The reasonably close agreement between these substances in corrected RSA values provided further evidence of the adequacy of the procedure, and also indicated the probability of the common origin of alcohol and acetate in these experiments. In all but the first experiment, the RSA was significantly below the theoretical value of 1.5 for exclusive operation of the Embden–Meyerhof process; hence it appears that the shunt, or other processes producing acetate from carbons other than glucose-1-carbon are occurring to some extent in these organisms. On the assumption that the E.M. and shunt processes account for all of the acetate production one can calculate from these RSA values that from 66 to 100% of the acetate arose via the E.M. process. From zero to as much as 17% of the C2 units formed by *S. cerevisiae* was derived *via* the shunt and *T. utilis* apparently produced from 20 to 35% of its C_2 compounds by this process. Though no systematic attempt was made to determine effects of conditions in this preliminary study, it appears that the relative extents of these processes are not altered greatly by changes in *p*H or by depletion of endogenous metabolites.

As a further critical test of this procedure, several experiments were conducted in which glucose-1-C^{14}, glucose-6-C^{14} and glucose-U-C^{14} were simultaneously catabolized by aliquots of the same yeast suspension. Reference to Fig. 1 reveals that 100% occurrence of the Embden–Meyerhof process would result in a yield from glucose-6-C^{14} of one labeled acetate molecule (from carbons 5 and 6) and one unlabeled (from carbons 1 and 2). As with glucose-1-C^{14}, therefore, a corrected RSA of 1.5 would be indicative of exclusive operation of the Embden–Meyerhof process. If the shunt operated exclusively, glucose-6-C^{14} would yield a single acetate molecule (from carbons 5 and 6); hence the acetate would have been a corrected RSA of 3.0. An element of uncertainty enters, however, with the possibility that in the operation of the shunt, C_2 molecules may arise from glucose carbons other than carbons 5 and 6. Studies of Gunsalus and Gibbs[12] with *Leuconostoc mesenteroides* and DeMoss[13] with *Pseudomonas lindneri* have shown that under certain circumstances C_2 units may arise from glucose carbons 2 and 3. On these grounds one can only set the value of 3.0 as an upper limit for exclusive operation of the shunt. However, it would appear reasonable to assume that a specific activity for acetate greater than 1.5 would be a good qualitative indication of the operation of the shunt.

Results of four such experiments are shown in Table III. In the anaerobic experiments with both yeast strains, closely

(12) I. C. Gunsalus and M. Gibbs, *J. Biol. Chem.*, **194**, 871 (1952).

(13) R. D. DeMoss, *J. Cellular Comp. Physiol.*, **41**, Suppl. 1, 207 (1953).

agreeing values, all close to 1.5, were obtained with glucose-1-C^{14} and glucose-6-C^{14}. Anaerobically, therefore, there is virtually exclusive operation of the E.M. process, though a minute occurrence of the shunt is betrayed by the appearance of some radioactivity from carbon 1 (but not from carbon 6) in the carbon dioxide. The aerobic experiments in Table III illustrate the uncertainties involved in the quantitative determination of relative extents of glucose catabolism pathways by this procedure. In the experiment with *S. cerevisiae*, the RSA of the acetate from glucose-1-C^{14} of 1.28 indicates that 85% of the acetate molecules are derived from the E.M. process; but the value of 1.55 for acetate from glucose-6-C^{14} indicates that (3–1.55) × 100/(3.0–1.50) = 96% has been so derived. Similarly, the experiment with *T. utilis*, though indicating a greater occurrence of the shunt, also displays a quantitative discrepancy between glucose-1-C^{14} and glucose-6-C^{14}. Whereas 75% occurrence of the E.M. process is indicated from the glucose-1-C^{14} data, a value of $(3.0-1.70) \times 100/3.0-1.50 =$ 87% is indicated from the glucose-6-C^{14} data. In view of the uncertainty concerning the number of acetate molecules arising from glucose *via* the shunt, we are inclined to accept the data from glucose-1-C^{14} as being more reliable than those from glucose-6-C^{14}. A more decisive figure must await results of similar experiments with glucose-2 or 3-C^{14}. Whatever the absolute value, however, the results indicate the shunt to be an important process in intact yeast cells. The markedly greater conversion of glucose-1-carbon to CO_2 also emphasizes the probability that an appreciable part of the glucose is being catabolized by a pathway in which this position is preferentially oxidized over the glucose-6-carbon.

TABLE III

CATABOLISM OF 6-C^{14} AND 1-C^{14} GLUCOSE BY YEAST

	Anaerobic experiments				Aerobic experiments			
	Cor. RSA		Total C_2 units produced *via* E.M. process, %		Cor. RSA		Total C_2 units produced *via* E.M. process, %	
	1-C^{14}	6-C^{14}	1-C^{14}	6-C^{14}	1-C^{14}	6-C^{14}	1-C^{14}	6-C^{14}
S. cerevisiae								
Ethanol	1.43	1.54	95	97	..	..	..	..
Acetate	..	..	..	..	1.28	1.55	85	96
Resp. CO_2	0.09	0.001	..	..	0.46	0.18	..	..
T. utilis								
Ethanol	1.48	1.60	98	94	1.13	1.70	75	87
Acetate	..	..	..	..	1.09	1.70	73	87
Resp. CO_2	0.09	0.002	..	..	0.76	0.097	..	..

Discussion

Results of the present study provide direct evidence of a process of glucose catabolism occurring aerobically in intact yeast cells, which is different from the glycolytic pathway of Embden and Meyerhof, and which is in accord with the hexose monophosphate shunt. On the assumption that the shunt represents the sole or major auxiliary mechanism of glucose catabolism the present method allows a calculation of the relative extents of each process. Despite the approximate nature of the values obtained, it is believed that the procedure provides a reliable order of magnitude of the occurrences of the E. M. and shunt processes. Anaerobically the E.M. process is greatly preponderant in the yeast strains studied; at least 95% of the C_2 units produced (either as acetate or ethanol) were so derived. These results are in accord with previous conclusions of Koshland and Westheimer[14] who found that all but 3% of the glucose-1-carbon fermented by baker's yeast was present in the methyl carbon of ethanol. Aerobically, however, in our experiments, an appreciable occurrence of the shunt was evident in both *S. cerevisiae* and *T. utilis*. Thus in five experiments with the former strain, from zero to as much as 17% of the C2 units were derived by the shunt, and in 6 experiments with *T. utilis*, from 25 to 33% were so derived.

The greater participation of the shunt during aerobiosis is in keeping with the idea that this process would be favored in the presence of oxygen since, in contrast with the balanced oxido-reductions of the E.M. process, the shunt requires dehydrogenations equivalent to the consumption of a mole of oxygen per mole of glucose. On this basis the greater occurrence of the shunt in the more highly aerobic *Torulopsis utilis* was not unexpected. However, there is no reason why the shunt could not occur anaerobically, since the oxidation reactions thereof could be coupled, through common coenzymes, with reductive reactions in the intact cell.

The term "percentage of total C2 units *via* E.M. process" requires some explanation. This value is not necessarily identical with the percentage of glucose molecules catabolized *via* this mechanism. The reason is that whereas the E.M. process yields two C2 units per glucose molecule catabolized, the shunt yields an as yet undetermined number between one and two. (Conceivably, it may yield even less than one C2 unit if much of the ribose is abstracted for nucleotide synthesis or other purposes). If we assume that the shunt yields one C2 unit we can calculate the relative proportions of glucose molecules undergoing the respective catabolic pathways as follows. From the relationships

Glucose catabolized *via* E.M. = $^1/_2$(C 2 units arising *via* E.M.)

Glucose catabolized *via* shunt = (C 2 units arising *via* shunt)

one can derive the following expression in terms of experimentally determined quantities.

$$\%\ \text{Glucose catabolized } \textit{via}\ \text{E.M.} = \frac{^1/_2(\%\ \text{C2 units } \textit{via}\ \text{E.M.}) \times 100}{^1/_2(\%\ \text{C2 units } \textit{via}\ \text{E.M.}) + (100 - \%\ \text{C2 units } \textit{via}\ \text{E.M.})}$$

which reduces to

$$\frac{\%\ \text{C2 units } \textit{via}\ \text{E.M. } (\times 100)}{200 - \%\ \text{C2 units } \textit{via}\ \text{E.M.}}$$

On the basis of these considerations the production of 80% of C2 units *via* the E.M. process is equivalent to the catabolism of 67% of the glucose molecules via this mechanism. Recalculating from the data of Table II, we find that from zero to 30% of the glucose molecules were catabolized via the shunt in *S. cerevisiae*, and from 32 to 50% were thus catabolized in *T. utilis*.

The possible effects of environmental conditions and strain differences on the relative participation of the two processes needs further investigation. The probability that these factors play a large part in the extent of occurrence of these two catabolic pathways is emphasized by other studies. Both

(14) D. E. Koshland, Jr., and F. H. Westheimer, THIS JOURNAL, **72**, 3383 (1950).

Gilvarg[15] and Sowden, *et al.*,[16] isolated mannose from polysaccharides of yeast grown on glucose-1-C^{14}. The former investigator working with *S. cerevisiae* found essentially the same activity in the mannose as in the original glucose, and the activity distribution indicated a direct conversion with little disruption of the carbon chain. In the same experiment, dilution of added acetate by acetate from glucose carbon was such as to indicate that essentially all of the acetate was derived via the E.M. process. In contrast, the latter investigators found no radioactivity in the mannose from *T. utilis*. This indicated that there was no direct conversion of glucose to mannose and that in this process carbon 1 of glucose was lost. The authors concluded from these results that the shunt was greatly preponderant in their organism.

In the present method of estimation the assumption is made that the E.M. process is the only one which yields labeled C2 units from glucose-1-C^{14}. Another possibility remains to be considered. Racker, de la Haba and Leder[17] recently reported that transketolase catalyzed the transfer of a "diose" from carbons one and two of fructose-6-phosphate to glyceraldehyde-3-phosphate or to ribose-5-phosphate, with the formation of the corresponding pentulose and heptulose phosphates. This would result in labeling of these sugars in the 1-position from glucose-1-C^{14}. Since the pentose thus labeled may add another "diose" to form a 1,3-labeled heptulose, and since these variously labeled heptuloses may transfer a triose moiety to another molecule of triose phosphate to form fructose-6-phosphate[18] a mechanism is apparent whereby labeling originally present in carbon 1 of glucose would appear in carbons 1 and 3 of fructose-6-phosphate. If these processes are rapid in comparison with the utilization of fructose-6-phosphate *via* the E.M. process, and if phosphohexose isomerase activity is sufficiently rapid, it is conceivable that glucose-6-phosphate would become labeled in carbon 3 and the further metabolism of this compound *via* the shunt would result in the formation of pentose labeled in carbon 2.

These reactions thus provide a means whereby labeled glucose carbon 1 can appear in pentose carbons 1 and 2. It is as yet unknown whether, in yeast, carbons 1 and 2 of pentose can yield acetate, as occurs in other organisms[12,13,19] or whether acetate may be formed from the "diose" split directly from fructose-6-phosphate. The possibility must be kept under consideration, therefore, that part of the C2 units calculated to have arisen *via* the E.M. pathway, in reality arose from "diose" units. This seems unlikely in the present experiments, however. If the "diose" pathway represented an important source of acetyl groups, we should expect a falsely high value for the E.M. pathway calculated from data obtained with glucose-1-C^{14}. However, somewhat lower values were obtained with glucose-1-C^{14} than were obtained with glucose-6-C^{14}, a result inconsistent with the formation of acetyl groups from glucose carbon 1 by a pathway other than that of Embden and Meyerhof.

(15) C. Gilvarg, *J. Biol. Chem.*, **199**, 57 (1952).

(16) J. C. Sowden, S. Frankel, B. H. Moore and J. E. McClary, *ibid.*, **206**, 547 (1954).

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The Constitution of Iles Mannan[1]

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Iles Mannan, the polysaccharide extracted from tubers of *Amorphophallus* plants is shown herein to be composed of two polysaccharides, one being a polyglucosan closely resembling amylose and the other a glucomannan. The latter is a linear polymer containing two parts of D-mannose and one part of D-glucose, the sugar residues being of the pyranose form and joined by 1,4-β-glycosidic bonds. The glucomannan exhibits retrogradation when aqueous solutions of it are heated. Precipitated in this manner, the glucomannan became insoluble in water and in alkali. It could be dissolved, however, in strong aqueous solutions of sodium xylenesulfonate. Prolonged drying of the glucomannan also rendered it insoluble in water and alkali.

Iles mannan meal is prepared from the tubers of the *Amorphophallus oncophyllus* and *Amorphophallus variabilis* plants which are native to and cultivated in Indonesia. It is a complex mixture containing a glucomannan and a smaller amount of a polyglucosan as its major carbohydrate constituents. Some cellulose, protein, lignin and minerals are also present in the meal. The mannose content is reported to vary from 43 to 75%, depending upon the species and growing conditions.[2,3] Although an aqueous solution of the mixture can be prepared, the glucomannan can be precipitated in a water-insoluble form by heating of its solution to 100°. Partly as a result of this peculiar solubility behavior it finds use in paper manufacture, as an adhesive and as a flocculating agent in rubber manufacture.[2]

This paper is concerned with the constitution of the glucomannan and the polyglucosan

(1) Extracted from a thesis submitted by P. A. Rebers to the University of Minnesota in partial fulfillment for the degree of Ph.D. (1953). Paper No. 3203, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

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