5. Some implications of these results are discussed.

We are grateful to Mr M. J. Hume and Miss M. Sandiford for expert technical assistance and to the Empire Rheumatism Council and the Board of Governors of King's College Hospital for grants towards the cost of the work.

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Uncoupling Reagents and Metabolism

2. EFFECTS OF 2:4-DINITROPHENOL AND SALICYLATE ON GLUCOSE METABOLISM IN BAKER'S YEAST

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(*Received* 29 *January* 1960)

Studies by Smith & Moses (1960) have shown that most, if not all, of the effects of salicylate and 2:4-dinitrophenol on intermediary metabolism in several animal tissues supplied with labelled glucose or acetate could be explained on the known abilities of these compounds to uncouple oxidative phosphorylation.

The present paper is concerned with similar experiments with baker's yeast. Studies were made of the kinetics of incorporation of the label into different metabolites, samples of the reaction mixtures being taken for analysis at time intervals ranging from a few seconds to several hours after the addition of the labelled substrate. A preliminary report of this work has already been published (Moses & Smith, 1960).

EXPERIMENTAL

Block yeast (3 g.) (The Distillers Co. Ltd.) was suspended in water and centrifuged at 1600 *g* for 5 min. The volume of the wet-packed cells was 3·8 ml. Half the cells (starved cells) were suspended in 25 ml. of tap water and the other half ('growing' cells) in 25 ml. of the following medium (g./l.); glucose, 10; NH_4Cl, 2; KH_2PO_4, 5·44; $MgSO_4,7H_2O$, 0·1; NaCl, 0·1, dissolved in London tap water [containing approximately (p.p.m.): Ca, 100; Na, 20–25; K, 5; Mg, 5 (all as bicarbonates); free Cl_2, less than 0·05] and adjusted to pH 5 with KOH. Each suspension was shaken at 25° for 3 hr., the cells were centrifuged, washed twice with 2 mM-KH_2PO_4, adjusted to pH 5 with KOH and resuspended in 19 ml. of this solution, which resulted in a cell concentration of 100 μl. of wet-packed cells/ml. When required, the final suspension also contained 2:4-dinitrophenol (DNP) or sodium salicylate at the appropriate concentration.

For the study of kinetics, 15 ml. of cell suspension was placed in a 50 ml. round-bottom flask equipped with a standard joint into which was fitted a Kipps automatic tilt pipette [H. J. Elliott Ltd. (E-Mil), Pontypridd, Glam.] calibrated to deliver 1 ml. The flask was shaken in air at 25° and the reaction was started, after removal of the tilt pipette, by the addition of 150 μl. of a solution of [^{14}C]-glucose (37·5 μc; 1·5 μmole; final glucose concn. 0·1 mM). [^{14}C]Glucose was obtained from The Radiochemical Centre, Amersham, Bucks.

The tilt pipette was replaced immediately and samples (1 ml.) of the suspension were run directly into 4 ml. of ethanol at room temperature at the following times after the addition of the labelled substrate: 5, 10, 15, 30, 45, 60,

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90, 120 and 180 sec.; 10, 30, 60 and 180 min. There was some unavoidable variation of the exact times at which the first three samples were taken, and these are recorded below in the Results section.

In experiments in which three samples only were required, 1 ml. of cell suspension was placed in each of three 25 ml. Erlenmeyer flasks, and the reaction initiated by the addition of 10 μl. of a solution of [^{14}C]glucose (2·5 μc; 0·1 μmole; final glucose concn., 0·1 mM). The flasks were shaken at 25° for 3, 30 and 180 min., and the reaction was terminated by the addition to the flask of 4 ml. of ethanol.

The aqueous ethanolic suspension of the cells was heated to boiling and the cells were separated by centrifuging at 1600 *g* for 5 min. The supernatant was removed and the cell residues were extracted again with 2 ml. of boiling aqueous 20% (v/v) ethanol. The extraction of the cells was repeated with 2 ml. of boiling water, the cell residues were discarded and the supernatants were pooled and concentrated to 0·6 ml. under reduced pressure at 40°. The radioactive substances in each extract were separated by paper chromatography, two chromatograms being run for each extract, one to detect all the substances in the extract and another to obtain a better separation of the phosphate esters. The techniques for chromatography, radioautography, spot identification and assay of radioactivity in each spot were the same as those described by Smith & Moses (1960).

RESULTS

Glucose metabolism in the absence of uncoupling reagents

The results of the incubation with [^{14}C]glucose of a suspension of yeast cells previously incubated for 3 hr. in a growth medium are shown in Table 1. The amount of radioactivity in each substance is expressed as a percentage of the sum of the radioactivity appearing in all the compounds listed; residual [^{14}C]glucose remaining in the reaction mixture, as well as that metabolized to $^{14}CO_2$, proteins, polysaccharides etc., has been excluded from the calculations.

Evidence was obtained for the occurrence in the yeast cells of several well-known metabolic pathways. The presence of radioactivity in glucose monophosphate, fructose monophosphate, fructose diphosphate, glucose diphosphate, phosphoglyceric acid and phosphoenolpyruvic acid supports the presence of the glycolytic sequence, and radioactivity in ribose monophosphate, sedoheptulose monophosphate and phosphogluconic acid indicates the operation of the pentose phosphate cycle. The presence of labelled phosphoglycollic acid and sedoheptulose diphosphate suggests the involvement of transketolase.

A number of radioactive citric acid-cycle intermediates were present (citric acid, fumaric acid, malic acid and succinic acid) as well as amino acids derived directly from citric acid-cycle intermediates by transamination (aspartic acid and glutamic acid). ^{14}C in alanine was probably derived by transamination from pyruvate. The presence of ^{14}C in glucose monophosphate, uridine diphosphoglucose and trehalose monophosphate supports the synthetic pathway for trehalose biosynthesis proposed by Leloir & Cabib (1953). Indeed trehalose was the only active disaccharide found, and at a late stage in the incubation more activity was found in this substance than in any other.

At the shortest incubation periods, most of the ^{14}C present in the soluble extract was confined to initial products of glucose metabolism (sugar phosphates) and relatively little radioactivity was found in substances such as amino acids and disaccharide; unchanged [^{14}C]glucose in the suspension gradually decreased in amount during the course of the experiment and had virtually disappeared after 3 hr. As the experiment progressed, lesser percentages of the radioactivity were found in the sugar phosphates, uridine diphosphoglucose and some organic acids, and ever greater percentages of the total ^{14}C appeared in the amino acids and trehalose. The radioactivity in the nucleotides remained low at all times.

Glucose metabolism in the presence of 2:4-dinitrophenol

A preliminary manometric experiment, to investigate the rate of endogenous oxygen uptake and the percentage of added glucose which was completely oxidized by the yeast, showed that at pH 5, 0·1 mM-DNP produced the greatest stimulation of the endogenous respiration and the greatest extent of complete oxidation of added glucose. This concentration of DNP was therefore used to study the effects of this substance on the metabolism of added [^{14}C]glucose by yeast previously suspended in growth medium for 3 hr.

Compared with results from the control experiment, the presence of DNP resulted in a decrease in the total ^{14}C found in the soluble extract of the cells, though the percentage of the total ^{14}C in the extract which was present in the sugar phosphates (particularly the hexose diphosphates, phosphoglyceric acid and phosphoenolpyruvic acid) remained relatively high for a longer period. The percentage of ^{14}C found in uridine diphosphoglucose, trehalose phosphate and free trehalose was much reduced, and no ^{14}C was found in trehalose phosphate until 30 sec., compared with 9 sec. for the control experiment. The percentages of ^{14}C in the various organic acids showed little variation from the control, but higher percentages were found in the amino acids, particularly in alanine, in which a high proportion of radioactivity was found throughout the experiment. Glutamic acid and aspartic acid contained a lower percentage of the total ^{14}C after the shorter incubation periods, and much higher percentages towards the end of the experiment (Table 2).

Table 1. *Kinetic study of* [^{14}C]*glucose metabolism by yeast*

Wet-packed cells (1·5 ml.), previously incubated in growth medium for 3 hr., were suspended in 15 ml. of 2 mM-KH_2PO_4 buffer, pH 5. [^{14}C]Glucose (37·5 μc, 1·5 μmole) was added and the suspension shaken at 25°. Samples (1 ml.) were removed as indicated below, the cells were killed and extracted with ethanol and the extracts chromatographed. The ^{14}C in each compound on the chromatogram was counted. The values given are expressed as the percentage of the total ^{14}C incorporated into all the soluble substances from labelled glucose, which is present in each individual substance. The ^{14}C remaining in the residual glucose was excluded from all calculations.

Incubation period (sec.)	9	12	18	30	45	60	90	120	180	600	1800	3600	10 800
Sugar diphosphates*	5·9	6·8	6·9	5·6	4·3	4·2	3·2	2·3	1·6	0·24	0·14	0·11	0·03
Sugar monophosphates†	37·6	42·2	42·5	39·4	33·5	26·1	22·0	16·7	12·0	2·4	0·60	1·3	1·2
Phosphoglyceric acid	1·2	1·5	2·3	2·9	3·7	4·3	4·9	4·9	5·4	2·0	0·90	0·48	0·38
Phosphogluconic acid	0·08	0·74	1·1	0·50	0·60	0·42	0·31	0·24	0·15	0·0	0·0	0·06	0·0
Trehalose phosphate	0·69	1·5	1·4	2·2	2·2	1·8	1·2	0·74	0·36	0·18	0·18	0·0	0·07
Phosphoenolpyruvic acid	1·1	0·56	0·85	1·1	1·2	1·4	2·1	2·6	2·6	0·69	0·39	0·21	0·15
Phosphoglycollic acid	0·29	0·33	0·30	0·30	0·17	0·20	0·09	0·12	0·15	0·0	0·08	0·10	0·10
Uridine diphosphoglucose	2·7	5·1	7·2	11·1	13·9	16·0	21·0	24·1	23·1	12·0	2·8	1·6	0·86
Uridine triphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·13	0·25	0·23	0·26	0·16
Guanosine diphosphate	0·0	0·0	0·0	0·0	0·0	0·07	0·0	0·13	0·21	0·28	0·61	0·32	0·26
Unidentified nucleoside monophosphate	0·0	0·98	0·0	0·0	0·0	0·21	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Unidentified nucleoside diphosphate	0·0	0·0	0·0	0·0	0·0	0·15	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Trehalose	3·1	3·4	5·8	10·3	14·3	17·4	20·5	29·0	29·7	43·4	53·7	58·8	62·2
Fructose	8·7	7·0	5·6	6·1	5·4	3·9	3·2	2·5	2·9	0·81	0·0	1·0	0·0
Cysteine	0·0	0·76	0·79	0·95	0·36	0·35	0·20	0·0	0·20	0·0	0·0	0·0	0·0
Aspartic acid	6·7	4·7	4·2	3·3	3·1	2·9	2·8	2·6	2·0	2·3	3·8	4·7	3·3
Glutamic acid	8·6	2·7	2·7	1·6	0·61	1·6	0·62	0·38	1·8	5·2	9·1	13·3	18·0
Alanine	0·0	2·6	2·9	4·6	5·4	6·5	6·0	4·6	6·1	20·2	19·7	10·8	7·5
Tyrosine	0·0	0·84	0·69	0·0	0·53	0·0	0·35	0·0	0·0	0·28	0·0	0·26	0·0
Glutamine	2·6	1·7	1·7	0·0	0·0	0·68	1·0	0·0	0·37	0·53	0·61	0·78	1·2
Phenylalanine + leucine	2·3	3·2	2·4	1·9	1·8	2·1	1·2	0·86	1·0	0·51	0·0	0·44	0·0
Valine	7·9	6·3	5·3	3·1	2·9	3·0	1·9	1·8	2·1	1·6	1·4	1·5	2·2
Citric acid	0·75	0·0	0·30	0·35	0·30	0·29	0·33	0·35	0·37	0·22	0·20	0·28	0·31
Fumaric acid	0·0	0·0	0·0	0·0	0·0	0·05	0·05	0·13	0·13	0·57	0·31	0·10	0·0
Malic acid	0·0	0·0	0·0	0·0	0·0	0·28	0·30	0·19	0·44	2·2	1·6	0·51	0·32
Succinic acid	2·6	0·88	0·38	0·46	0·56	1·3	0·76	0·30	0·94	1·1	0·34	0·64	0·0
Glyceric acid	4·4	5·6	3·9	3·6	5·0	3·0	5·3	5·3	6·1	2·9	2·7	1·7	1·5
Unidentified substances	2·6	0·64	0·78	0·55	0·22	1·7	0·11	0·09	0·23	0·15	0·52	0·80	0·34
Total in sugar phosphates	46·9	53·6	55·2	52·1	45·8	38·4	34·4	27·7	22·2	5·6	2·6	2·3	1·9
Total in uridine diphosphoglucose	2·7	5·1	7·2	11·1	13·9	16·0	21·0	24·1	23·1	12·0	2·8	1·6	0·86
Total in nucleotides	0·0	0·98	0·0	0·0	0·0	0·42	0·0	0·13	0·34	0·53	0·84	0·58	0·42
Total in free sugars	11·8	10·5	11·4	16·4	19·7	21·3	23·6	31·5	32·6	44·2	53·7	59·8	62·2
Total in amino acids	28·1	22·8	20·6	15·5	14·7	17·2	14·1	10·2	13·6	30·6	34·6	31·8	32·1
Total in organic acids	7·8	6·5	4·5	4·4	5·8	5·0	6·8	6·3	7·9	6·9	5·2	3·2	2·1
10^{-5} × Total activity (counts/min./100 μl. of cells)	0·34	0·62	0·71	1·03	1·19	1·51	1·65	1·86	2·25	2·12	1·97	2·08	1·89

* Contains diphosphates of glucose, fructose and sedoheptulose.

† Contains monophosphates of glucose, fructose, sedoheptulose and ribose.

Table 2. *Kinetic study of* [^{14}C]*glucose metabolism by yeast in the presence of* 2:4-*dinitrophenol*

Experimental conditions were the same as given for Table 1, except that the reaction mixture during incubation with [^{14}C]glucose contained 0·1 mM-2:4-dinitrophenol.

Incubation period (sec.)	6	12	15	30	45	60	90	120	180	600	1800	3600	10 800
Sugar diphosphates*	8·3	10·8	9·7	4·8	2·2	2·8	1·8	6·1	2·0	0·20	0·34	0·19	0·0
Sugar monophosphates†	31·4	42·9	40·8	32·5	26·5	26·3	22·6	17·5	16·7	1·9	1·3	0·74	0·94
Phosphoglyceric acid	7·4	14·0	13·8	13·2	12·5	16·9	14·0	13·2	8·5	0·78	0·12	0·22	0·27
Phosphogluconic acid	0·64	0·87	0·40	0·47	0·20	0·35	0·32	0·32	0·19	0·0	0·0	0·0	0·0
Trehalose phosphate	0·0	0·0	0·0	0·08	0·14	0·10	0·23	0·0	0·08	0·0	0·0	0·0	0·0
Phosphoenolpyruvic acid	3·3	1·1	1·8	4·6	4·0	3·3	3·8	3·8	2·6	0·47	0·11	0·01	0·05
Phosphoglycollic acid	0·0	0·0	0·0	0·0	0·0	0·10	0·0	0·25	0·07	0·13	0·0	0·12	0·14
Uridine diphosphoglucose	0·71	1·3	1·6	2·1	2·6	3·8	5·9	6·7	8·4	8·8	4·3	2·8	1·3
Uridine triphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·30	0·17	0·21	0·24
Guanosine diphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·06	0·55	0·13	0·77
Unidentified nucleoside monophosphate	0·0	1·1	1·1	0·28	0·14	0·09	0·63	0·56	0·34	0·12	0·30	0·03	0·0
Unidentified nucleoside diphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·13	0·0	0·26	0·0
Trehalose	1·1	0·69	4·2	3·0	2·7	2·4	1·4	1·9	2·6	3·2	4·0	4·3	5·8
Fructose	14·0	4·9	3·8	3·9	4·0	2·7	2·1	0·93	1·3	0·0	0·0	0·0	0·0
Cysteine	0·31	0·0	0·26	0·0	0·26	0·0	0·20	0·0	0·11	0·0	0·16	0·0	0·0
Aspartic acid	5·0	2·5	1·5	1·8	1·5	1·1	2·0	1·4	1·6	2·1	2·1	2·8	15·0
Glutamic acid	0·0	0·0	0·0	0·56	1·1	1·7	3·0	4·6	6·3	17·5	34·2	47·7	48·7
Alanine	12·3	13·5	13·9	24·8	31·2	30·6	29·6	33·5	38·9	55·8	42·3	29·8	15·3
Tyrosine	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·16	0·0	0·0	0·0	0·07	0·0
Glutamine	0·0	0·0	0·0	0·0	0·0	0·0	1·8	1·7	0·56	0·0	0·94	1·7	2·9
Phenylalanine + leucine	1·7	0·0	0·73	0·49	1·0	0·20	0·74	0·08	0·08	0·08	0·10	0·04	0·0
Valine	3·7	1·8	1·5	0·58	0·65	0·46	0·46	0·86	1·6	1·9	1·6	1·1	1·2
Citric acid	0·0	0·16	0·25	0·38	0·57	0·36	0·74	0·75	0·85	0·47	0·73	0·62	0·38
Fumaric acid	0·0	0·0	0·0	0·0	0·0	0·06	0·10	0·23	0·41	0·67	0·88	0·46	0·28
Malic acid	0·0	0·0	0·0	0·0	0·15	0·45	0·31	0·70	1·4	1·6	2·4	2·3	1·0
Succinic acid	1·1	0·0	0·53	0·67	0·54	0·63	0·15	0·90	0·96	1·0	0·79	0·88	1·0
Glyceric acid	6·1	4·4	4·3	5·4	7·9	5·1	6·8	2·6	2·4	0·32	0·28	0·10	0·17
Unidentified substances	2·9	0·0	0·0	0·59	0·0	0·55	1·6	1·4	2·2	2·5	2·4	3·2	4·6
Total in sugar phosphates	51·1	69·7	66·4	55·6	45·6	49·9	42·4	41·1	30·1	3·5	1·9	1·3	1·4
Total in uridine diphosphoglucose	0·71	1·3	1·6	2·1	2·6	3·8	5·9	6·7	8·4	8·8	4·3	2·8	1·3
Total in nucleotides	0·0	1·1	1·1	0·28	0·14	0·09	0·63	0·56	0·34	0·51	1·0	0·63	1·0
Total in free sugars	15·1	5·6	8·0	6·9	6·7	5·1	3·5	2·8	3·9	3·2	4·0	4·3	5·8
Total in amino acids	23·0	17·7	17·9	28·2	35·7	34·1	37·8	42·3	29·1	77·4	81·1	83·5	82·9
Total in organic acids	7·2	4·6	5·1	6·5	9·1	6·6	8·1	5·2	6·0	4·1	5·0	4·4	2·9
10^{-5} × Total activity (counts/min./100 μl. of cells)	0·19	0·24	0·26	0·53	0·72	0·74	0·75	0·93	0·98	0·93	1·10	1·14	0·88

* Contains diphosphates of glucose, fructose and sedoheptulose.

† Contains monophosphates of glucose, fructose, sedoheptulose and ribose.

Table 3. *Kinetic study of* [^{14}C]*glucose metabolism by yeast in the presence of sodium salicylate*

Experimental conditions were the same as given for Table 1, except that the reaction mixture during incubation with [^{14}C]glucose contained 8 mM-sodium salicylate.

Incubation period (sec.)	6	11	15	30	45	60	90	120	180	600	1800	3600	10 800
Sugar diphosphates*	0·94	1·6	2·1	2·7	2·2	2·1	2·0	1·2	1·0	0·18	0·0	0·0	0·0
Sugar monophosphates†	17·2	22·2	27·6	30·7	29·5	27·4	25·3	19·7	9·8	2·1	1·0	1·5	1·6
Phosphoglyceric acid	1·6	1·1	1·7	4·3	5·8	4·1	3·7	3·4	2·4	0·84	0·20	0·16	0·24
Phosphogluconic acid	0·32	0·77	0·48	0·47	0·42	0·27	0·46	0·05	0·22	0·04	0·06	0·09	0·08
Trehalose phosphate	0·0	0·0	0·0	0·28	0·24	0·50	0·39	0·45	0·18	0·22	0·21	0·0	0·0
Phosphoenolpyruvic acid	0·59	0·64	0·86	0·90	1·0	0·90	0·92	0·82	0·93	0·21	0·09	0·10	0·16
Phosphoglycollic acid	0·0	1·3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Uridine diphosphoglucose	0·64	1·4	2·1	3·5	4·8	6·4	9·7	10·8	13·9	10·1	4·5	3·6	1·5
Uridine triphosphate	0·0	0·0	0·96	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·05	0·27	0·25
Guanosine diphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·16	0·24	0·37	0·72	0·32
Unidentified nucleoside monophosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·32	0·0	0·30
Unidentified nucleoside diphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·13	0·15	0·16	0·17
Trehalose	5·8	5·7	3·4	4·7	3·7	3·2	7·1	7·6	8·8	9·8	11·7	12·9	16·2
Fructose	13·3	11·6	13·9	11·8	13·0	11·9	11·8	11·6	6·2	1·9	0·60	0·0	0·0
Cysteine	1·5	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Aspartic acid	13·7	10·7	9·6	7·9	5·6	4·0	4·2	4·3	3·3	2·6	3·1	7·9	9·2
Glutamic acid	6·6	5·7	4·1	3·6	2·4	3·0	2·2	2·7	6·9	17·8	34·3	21·5	40·7
Alanine	7·4	4·9	6·2	7·2	9·7	12·1	8·9	14·7	21·9	39·4	29·5	33·8	16·9
Tyrosine	0·0	1·6	1·4	0·0	0·50	0·41	0·91	0·53	0·75	0·23	0·0	0·0	0·0
Glutamine	0·0	2·8	0·0	0·0	0·0	0·0	0·78	0·0	0·0	0·0	0·59	1·3	2·7
Phenylalanine + leucine	4·6	3·9	4·3	3·2	2·7	2·5	2·1	3·1	1·6	0·64	0·93	1·4	0·78
Glutathione	0·0	2·3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Valine	7·4	6·4	5·9	4·1	3·0	3·6	4·1	4·1	3·5	1·7	1·5	2·3	1·7
Citric acid	0·0	0·0	0·41	0·34	0·81	1·4	2·8	3·1	5·3	2·6	0·83	0·78	0·50
Fumaric acid	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·26	0·51	0·60	0·90	0·23
Malic acid	0·0	0·0	0·0	0·18	0·23	0·13	0·34	0·40	0·97	2·0	2·8	3·2	1·1
Succinic acid	0·68	0·24	0·30	0·0	0·20	0·90	1·1	0·30	0·52	2·3	2·3	2·1	1·4
Glyceric acid	9·7	9·4	8·1	10·7	12·1	11·8	10·3	11·0	10·7	4·3	1·6	1·6	1·8
Unidentified substances	8·0	5·6	6·7	3·6	2·0	2·0	0·90	0·41	0·67	0·12	2·7	3·3	1·9
Total in sugar phosphates	20·6	27·8	32·6	39·1	39·3	35·3	32·7	25·6	14·5	3·7	1·6	1·8	2·0
Total in uridine diphosphoglucose	0·64	1·4	2·1	3·5	4·8	6·4	9·7	10·8	13·9	10·1	4·5	3·6	1·5
Total in nucleotides	0·0	0·0	0·96	0·0	0·0	0·0	0·0	0·0	0·16	0·37	0·89	1·2	1·0
Total in free sugars	19·1	17·3	17·3	16·5	16·7	15·1	18·9	19·2	15·0	11·7	12·3	12·9	16·2
Total in amino acids	41·2	38·3	31·5	26·0	23·9	27·2	23·1	29·5	37·9	62·4	69·9	68·5	72·2
Total in organic acids	10·4	9·6	8·9	11·2	13·3	14·2	14·6	14·9	17·8	11·6	8·2	8·5	5·1
10^{-5} × Total activity (counts/min./100μl. of cells)	0·25	0·28	0·39	0·55	0·74	0·82	0·82	1·02	1·12	1·22	1·50	1·16	1·29

* Contains diphosphates of glucose, fructose and sedoheptulose.

† Contains monophosphates of glucose, fructose, sedoheptulose and ribose.

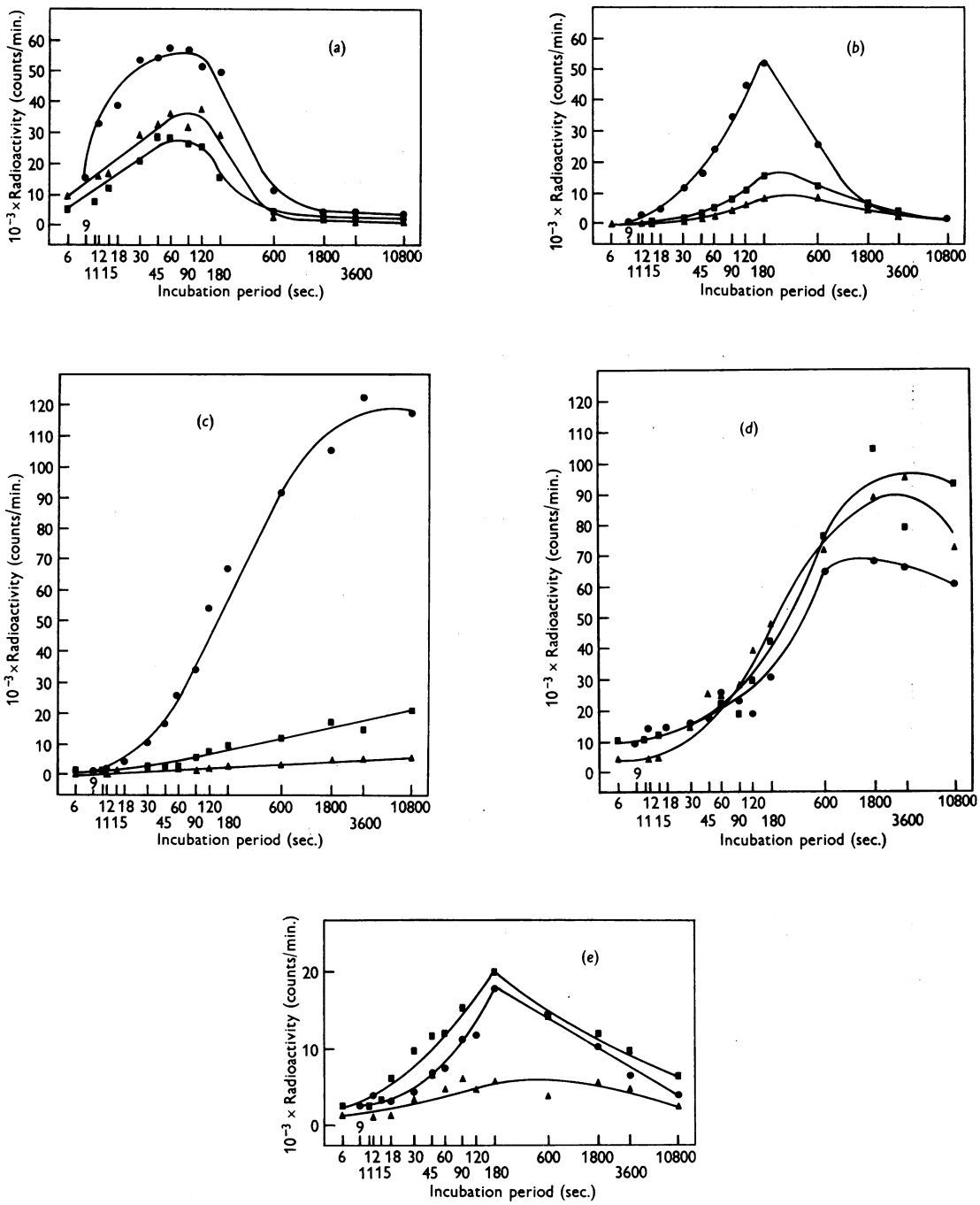

Fig. 1. Incorporation of ^{14}C from [^{14}C]glucose into various groups of compounds by baker's yeast. Experimental details were as given for Tables 1–3. Results are plotted as counts/min./100 μl. of wet-packed cells of the ^{14}C incorporated into each group of compounds. (*a*) Sugar phosphates, (*b*) uridine diphosphoglucose, (*c*) trehalose, (*d*) amino acids, (*e*) organic acids. ●, Control with no uncoupling reagent; ▲, with 0·1 mM-DNP; ■, with 8 mM-salicylate.

If the values recorded in Tables 1 and 2 are expressed as the actual quantities of ^{14}C present in each compound, rather than as a percentage of the total soluble ^{14}C, it was found that, compared with the controls, cells incubated in the presence of DNP contained less radioactivity in the sugar phosphates, uridine diphosphoglucose and trehalose, and more in the amino and organic acids. Little change was observed in the nucleotide fraction (Fig. 1).

Glucose metabolism in the presence of sodium salicylate

A manometric experiment similar to that mentioned above showed that, at pH 5, 10 mM-salicylate produced the maximum stimulation of the endogenous respiration of the yeast and caused the greatest oxidation of added glucose. However, as the rates of respiration, both with and without added glucose, fell very rapidly at concentrations of salicylate higher than 10 mM, a concentration of 8 mM was used to avoid the possibility of inhibiting respiratory processes.

Table 3 shows the results of incubating yeast cells with [^{14}C]glucose in the presence of 8 mM-sodium salicylate. Expressed on a percentage basis, sodium salicylate had a similar effect to DNP on the distribution of ^{14}C among the products of glucose metabolism, with the following minor differences: compared with the controls incubated in the absence of uncoupling reagents, salicylate-

Table 4. *Effect of high concentrations of 2:4-dinitrophenol and sodium salicylate on [^{14}C]glucose metabolism in yeast*

Each flask contained 0·1 ml. of wet-packed cells, previously incubated in growth medium for 3 hr., suspended in 1 ml. of 2 mM-KH_2PO_4 buffer, pH 5. [^{14}C]Glucose (2·5 μc, 0·1 μmole) was added and the suspension incubated at 25°. When present, the concn. of 2:4-dinitrophenol was 1 mM, and that of sodium salicylate, 50 mM. The cells were killed after the appropriate incubation period by the addition of 4 ml. of ethanol. Other details were as given for Table 1.

Uncoupling agent	None			2:4-Dinitrophenol			Sodium salicylate		
Incubation period (sec.) ...	180	1800	10 800	180	1800	10 800	180	1800	10 800
Sugar diphosphates*	0·65	0·18	0·34	0·70	0·0	0·0	0·72	0·0	0·0
Sugar monophosphates†	15·3	1·7	1·9	4·4	2·4	0·90	18·0	0·80	0·79
Phosphoglyceric acid	7·0	2·3	0·47	7·4	0·0	0·24	5·4	0·56	0·38
Phosphogluconic acid	0·46	0·03	0·51	0·27	0·15	0·05	0·43	0·17	0·0
Trehalose phosphate	0·56	0·08	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Phosphoenolpyruvic acid	3·6	4·2	0·12	4·8	0·51	0·98	4·4	1·9	0·74
Uridine diphosphoglucose	3·3	0·44	2·0	0·89	2·0	0·13	0·82	3·2	1·5
Uridine triphosphate	0·10	0·15	0·10	0·0	0·0	0·03	0·0	0·24	0·44
Guanosine diphosphate	0·21	0·24	0·87	0·0	0·0	0·0	0·0	0·15	0·29
Adenosine triphosphate	0·0	0·14	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Unidentified nucleoside monophosphate	0·44	0·10	0·45	0·0	0·0	0·0	0·0	0·96	0·56
Trehalose	31·7	49·3	51·3	1·9	1·4	0·86	8·5	8·5	11·9
Fructose	2·0	0·0	0·0	0·0	0·0	1·4	8·0	0·0	2·1
Aspartic acid	1·9	3·8	3·3	4·0	3·7	2·3	4·4	3·2	3·3
Glutamic acid	1·2	10·1	17·1	13·7	32·5	54·3	2·2	18·0	30·1
Alanine	3·3	14·3	10·1	28·6	39·1	20·7	12·9	28·3	23·9
Tyrosine	0·0	0·42	1·0	3·5	1·9	1·1	0·0	0·0	0·0
Glutamine	0·72	0·21	1·3	2·1	0·79	1·7	3·5	1·2	1·2
Phenylalanine + leucine	1·3	1·8	1·2	3·2	1·6	1·2	1·6	3·4	1·2
Valine	1·2	0·94	2·2	4·0	3·0	2·7	4·8	3·3	4·1
Citric acid	8·7	2·7	0·17	3·2	0·35	0·69	3·9	6·5	0·54
Fumaric acid	1·1	0·40	0·06	0·28	0·18	0·13	0·0	0·66	0·29
Malic acid	0·84	0·59	0·27	1·3	1·7	0·95	0·93	1·6	0·94
Succinic acid	6·3	2·2	0·34	8·9	0·0	1·4	8·9	2·7	1·6
Glyceric acid	4·2	1·1	2·0	3·0	4·4	1·7	5·0	4·8	4·0
Unidentified substances	4·2	2·6	3·1	4·0	4·3	6·5	5·5	9·8	10·1
Total in sugar phosphates	27·5	8·4	3·3	17·6	3·1	2·1	29·0	3·5	1·9
Total in uridine diphosphoglucose	3·3	0·44	2·0	0·89	2·0	0·13	0·82	3·2	1·5
Total in nucleotides	0·75	0·63	1·4	0·0	0·0	0·03	0·0	1·3	1·3
Total in free sugars	33·7	49·3	51·3	1·9	1·4	2·3	16·5	8·5	14·0
Total in amino acids	9·5	31·6	36·1	59·0	82·6	84·0	29·4	57·3	63·8
Total in organic acids	21·1	7·1	2·8	16·7	6·7	4·9	18·8	16·3	7·3
10^{-5} × Total activity (counts/min./100 μl. of cells)	2·52	2·25	1·69	0·76	1·04	1·22	0·81	0·83	1·02

* Contains diphosphates of glucose, fructose and sedoheptulose.

† Contains monophosphates of glucose, fructose, sedoheptulose and ribose.

treated cells showed a smaller percentage incorporation of ^{14}C into the sugar phosphates (particularly the monophosphates, diphosphates, phosphogluconic acid and phosphoenolpyruvic acid) and a greater incorporation into glyceric acid, and into aspartic acid, and alanine throughout the course of the experiment. However, the percentage incorporation into alanine was not as high as in the presence of DNP.

On the basis of the absolute radioactivity in each compound, the results with salicylate compared with those from controls are similar to those with DNP except that with salicylate more activity was found in the organic acids (Fig. 1). When the absolute activities obtained in the presence of salicylate are compared with those found in the presence of DNP, salicylate-treated cells had more activity in uridine diphosphoglucose, trehalose and the organic acids, and about the same in the nucleotides and in the soluble extract as a whole. However, less radioactivity was found in the sugar phosphates (Fig. 1).

Table 5. *Effect of 2:4-dinitrophenol and sodium salicylate on [^{14}C]glucose metabolism in starved yeast*

Experimental conditions were as given in Table 4, except that the yeast cells were previously starved by incubation for 3 hr. at 25° in 2 mM-KH_2PO_4 buffer, pH 5. The concentrations of 2:4-dinitrophenol and sodium salicylate in the incubation medium were 0·1 mM and 8 mM respectively.

Uncoupling agent	None			2:4-Dinitrophenol			Sodium salicylate		
Incubation period (sec.) ...	180	1800	10 800	180	1800	10 800	180	1800	10 800
Sugar diphosphates*	1·6	0·08	0·0	0·95	0·06	0·0	0·73	0·04	0·0
Sugar monophosphates†	15·4	2·2	2·7	14·7	4·7	0·80	7·6	1·5	1·6
Phosphoglyceric acid	6·4	2·3	0·68	3·8	1·3	0·0	2·7	0·59	0·22
Phosphogluconic acid	0·0	0·0	0·0	0·30	0·0	0·0	0·22	0·0	0·08
Trehalose phosphate	0·40	0·0	0·0	0·13	0·0	0·0	0·47	0·0	0·0
Phosphoenolpyruvic acid	1·6	0·58	0·16	3·0	0·24	0·07	0·81	0·15	0·04
Phosphoglycollic acid	0·25	0·11	0·20	0·0	0·0	0·0	0·0	0·0	0·0
Uridine diphosphoglucose	22·8	11·2	6·2	8·9	0·26	1·7	16·4	6·5	2·8
Uridine triphosphate	0·15	0·40	0·46	0·0	0·0	0·14	0·0	0·25	0·44
Guanosine diphosphate	0·32	1·0	1·4	0·13	0·37	0·56	0·23	0·48	0·24
Unidentified nucleoside monophosphate	0·0	0·0	0·0	0·27	0·0	0·0	0·0	0·29	0·0
Unidentified nucleoside diphosphate	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·73
Trehalose	19·3	31·8	37·8	1·7	3·3	7·0	9·4	13·0	17·8
Fructose	3·4	0·0	0·0	2·8	0·15	0·0	7·3	1·4	1·3
Cysteine	0·0	0·0	0·0	0·04	0·0	0·0	0·0	0·0	0·0
Aspartic acid	2·8	5·4	5·5	2·0	8·6	11·6	4·1	5·4	7·2
Glutamic acid	1·8	16·5	23·2	10·9	37·0	50·7	8·1	31·1	38·3
Alanine	7·4	18·8	11·1	26·3	36·7	20·5	15·2	24·8	17·1
Tyrosine	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·34	0·25
Glutamine	1·1	0·84	1·0	1·4	0·30	0·97	0·0	1·1	1·1
Phenylalanine + leucine	1·6	1·0	1·1	0·93	0·72	0·75	1·5	1·3	1·1
Glutathione	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·12	0·19
Valine	2·0	1·3	1·3	0·92	0·12	0·33	2·9	1·7	2·2
Citric acid	0·40	1·2	2·4	1·1	1·0	1·2	3·6	1·7	2·0
Fumaric acid	0·0	0·0	0·0	0·30	0·37	0·08	0·17	0·27	0·09
Malic acid	0·0	0·27	0·37	0·0	0·0	0·42	0·76	1·4	0·48
Succinic acid	0·0	0·21	0·31	1·1	1·2	1·2	0·50	0·34	0·53
Glyceric acid	11·3	4·5	1·7	18·1	2·6	0·22	17·5	3·6	1·8
Unidentified substances	0·0	0·24	2·2	0·57	1·1	1·8	0·24	2·3	2·6
Total in sugar phosphates	25·7	5·3	3·7	22·9	6·3	0·87	12·5	2·3	1·9
Total in uridine diphosphoglucose	22·8	11·2	6·2	8·9	0·26	1·7	16·4	6·5	2·8
Total in nucleotides	0·47	1·4	1·9	0·40	0·37	0·70	0·23	1·0	1·4
Total in free sugars	22·7	31·8	37·8	4·5	3·5	7·0	16·7	14·4	19·3
Total in amino acids	16·6	43·8	43·4	42·4	83·3	84·9	31·7	66·3	67·9
Total in organic acids	11·7	6·2	4·8	20·6	5·2	3·1	22·5	7·4	4·8
10^{-5} × Total activity (counts/min/100 µl. of cells)	1·86	2·03	1·59	1·07	1·71	1·12	1·14	2·08	1·77

* Contains diphosphates of glucose, fructose and sedoheptulose.
† Contains monophosphates of glucose, fructose, sedoheptulose and ribose.

Effects of high concentration of uncoupling reagents on [^{14}C]glucose metabolism

Further experiments were performed to investigate the effects on labelled glucose metabolism of concentrations of DNP (1 mM) and salicylate (50 mM) that were known from preliminary manometric data to be inhibitory both to the endogenous respiration of the yeast and to the rate of oxygen uptake with glucose as substrate. Three incubation periods only were used: 3, 30 and 180 min.

The changes in percentage distribution in the presence of the higher concentrations of uncouplers did not differ from those produced by the lower concentrations: there was, however, a small decrease in the total amount of ^{14}C incorporated into the soluble extract (Table 4).

Effect of uncoupling agents on [^{14}C]glucose metabolism in starved cells

Starved cells were incubated with labelled glucose in the presence and absence of 0·1 mM-DNP or 8 mM-sodium salicylate for 3, 30 and 180 min. (Table 5). The experiments were performed at the same times as those reported in Tables 1–3 and used the same starting material (see Experimental section).

In the absence of uncoupling reagents, the main effects of starvation (cf. Table 1) were to reduce the total ^{14}C incorporated into the soluble extract, and to reduce the percentage radioactivity found in trehalose. However, a higher percentage of the radioactivity was found in uridine diphosphoglucose, the nucleotides and glyceric acid at the longer incubation periods, and in the amino acids, sugar monophosphates and phosphoglyceric acid during the whole incubation.

In the presence of the uncoupling reagents, compared with the appropriate experiments with unstarved cells (Tables 2 and 3), the effect of starvation was to increase the total soluble ^{14}C and to cause some alteration in the percentage distribution among the various amino acids.

DISCUSSION

Routes of glucose metabolism in baker's yeast

The present studies have confirmed the work of Aubert & Milhaud (1955), who found ^{14}C in a number of sugar phosphates, amino acids, organic acids, uridine diphosphate derivatives and trehalose when yeast was incubated with labelled glucose. Glucose is thus metabolized through glycolysis, the pentose phosphate cycle, the citric acid cycle and via uridine diphosphoglucose to trehalose phosphate and free trehalose. It was impossible to estimate in the present work either the relative importance of the glycolytic and hexose monophosphate-shunt mechanisms, or the significance of the citric acid cycle as an oxidative pathway rather than a synthetic mechanism for the production of amino acids. Beevers & Gibbs (1954), however, suggested that the pentose phosphate cycle may be the main route of glucose breakdown, and Krebs, Gurin & Eggleston (1952) believe that the principal function of the citric acid cycle is for synthesis and not for the oxidation of C_2 fragments to carbon dioxide.

The incorporation of ^{14}C into all the substances investigated can be explained by well-established biochemical pathways. However, the role in metabolism of free fructose and free glyceric acid, both of which contained appreciable amounts of radioactivity, is not clear. Chromatographic analysis of the glucose revealed traces of fructose. Alternatively it is possible that yeast forms and subsequently utilizes free fructose, or that fructose is formed from a fructose phosphate by the momentary action of a phosphatase during the killing procedure with ethanol. Although glyceric acid was not a contaminant in the [^{14}C]glucose, whether its presence indicates that it was a genuine metabolic intermediate, possibly on the pathway of glycine and serine synthesis, or whether it may also have been formed by hydrolysis of a phosphate precursor, is uncertain.

Effects of uncoupling reagents

Although Lutwak-Mann (1942) found little effect of salicylate on the respiration of yeast at a concentration less than 0·1 M, the present studies have demonstrated effects on the uptake and distribution of ^{14}C from [^{14}C]glucose by both 0·1 mM-DNP and 8 mM-sodium salicylate. In addition to a number of minor variations between experiments, the predominant effect of the uncoupling reagents for the longer incubation periods (30–180 min.) was to suppress the absolute incorporation of ^{14}C into all classes of compounds except the amino acids, into which the incorporation was increased: the inhibition of incorporation of ^{14}C was most marked with trehalose. At shorter incubation periods (30–90 sec.) the overall picture was similar, except that the most marked inhibition was into uridine diphosphoglucose, trehalose phosphate and trehalose, and the incorporation into amino acids was either decreased or slightly stimulated (Fig. 1). Thus the observable effects of uncoupling reagents depend on the time at which the observation is made. In the early stages the incorporation of ^{14}C into the intermediates of certain metabolic sequences was inhibited, whereas during the later stages the inhibition was more pronounced in relation to the end products of those reaction sequences.

A comparison with the effect of an uncoupler of oxidative phosphorylation in another organism is provided by the observation that in starved *Zygorrhynchus moelleri* the incorporation of ^{14}C from [^{14}C]glucose into all classes of compounds except amino and organic acids was inhibited by 1 mM-sodium azide, with the most prominent inhibition affecting the nucleotides and maltose (Moses, 1959).

Most of the effects of DNP and salicylate are explicable on the basis of both of these substances being primarily uncouplers of oxidative phosphorylation and hence blocking the supply of energy for synthetic purposes. The more important compounds requiring energy for synthesis are uridine diphosphoglucose, trehalose, polysaccharides, nucleotides and proteins, and if their synthesis is prevented by an insufficiency of adenosine triphosphate (ATP) it may be expected that the compounds immediately preceding the blocked steps would accumulate unless there were alternative metabolic routes available for them to escape. Of the compounds requiring energy for synthesis, only uridine diphosphoglucose and trehalose have been directly measured. In the absence of uncouplers, a large amount of radioactivity appeared in trehalose after 3 hr., whereas very little appeared in the presence of DNP and salicylate. Glucose and ribose phosphates might be expected to accumulate, but these compounds can be converted through glycolysis or the pentose phosphate cycle into pyruvate and may not accumulate for this reason. The relative accumulation of amino acids and organic acids in the presence of the uncouplers is probably due to the fact that the citric acid cycle is a pathway primarily for amino acid synthesis and not for oxidation (Krebs *et al.* 1952), and the mechanisms for organic acid and amino acid oxidation to carbon dioxide may not operate at a sufficient rate to reduce the accumulation of amino acids.

In the restricted view of cellular metabolism that these experiments provides, no specific effects of salicylate have been found distinct from those of DNP, except that salicylate tends to be rather weaker in its action even at the most effective concentration for each uncoupler. Both substances appear from these studies to act solely as uncouplers of oxidative phosphorylation, a conclusion which also applies to the studies with animal tissues (Smith & Moses, 1960).

SUMMARY

1. Studies have been made of the metabolism of [^{14}C]glucose by baker's yeast previously suspended for 3 hr. in growth medium. Samples of the reaction mixture were taken at intervals from about 5 sec. to 3 hr. ^{14}C was incorporated into a number of compounds, which suggests that glucose is metabolized through glycolysis, the pentose phosphate cycle, the citric acid cycle and via uridine diphosphoglucose to trehalose phosphate and free trehalose.

2. Similar experiments performed in the presence of 0·1 mM-2:4-dinitrophenol and 8 mM-sodium salicylate showed that the main effect of these uncouplers of oxidative phosphorylation was to suppress partially the incorporation of ^{14}C into all compounds except the amino acids; the inhibition was most marked with uridine diphosphoglucose and trehalose.

3. The principal result of increasing the concentration of the uncouplers to 1 mM-2:4-dinitrophenol and 50 mM-salicylate was to depress still further the total incorporation of ^{14}C, though the distributional pattern of the isotope in a number of substances was similar to that with the lower concentrations.

4. Starving the yeast cells before the addition of [^{14}C]glucose resulted in a fall of ^{14}C incorporated into trehalose, and a rise in all other fractions except the organic acids. In experiments with the uncoupling agents, starvation resulted in an increase in the total ^{14}C taken up into the soluble extract of the cells and some distributional variation among the amino acids.

5. The effects of 2:4-dinitrophenol and salicylate on [^{14}C]glucose metabolism are explicable on the basis of their uncoupling action. Little difference was observed between the action of 2:4-dinitrophenol and that of salicylate.

We are grateful to the Empire Rheumatism Council and to the Board of Governors of King's College Hospital for grants towards the cost of this work. We would also like to express our thanks to Mr M. J. Hume for technical assistance.

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