The phosphorylation accompanying oxidation of either substrate was sensitive to dinitrophenol as shown in the table. As in the work of TAPLEY *et al.*[8] on fragments obtained by digitonin treatment of mitochondria, our material was not sensitive to thyroxin.

The final suspension of the enzyme may be made in water rather than in the fluoride-phosphate solution indicated above. Under these circumstances the preparation exhibits a very marked dependence on phosphate concentration. The table indicates that Mg was an obligate component for the coupled phosphorylation. While Mg has generally been assumed to be essential we believe this demonstration to be the first definite evidence for it. Different levels of Mg were employed for the two substrates, however, the requirement was found to be the same in the two cases. In the absence of Mg, no labelling of any sort occurred.

Although our preparations have β-hydroxybutyric dehydrogenase, no oxidation of β-hydroxybutyrate occurs without added DPN^+, in which case the P:O ratios are similar to those obtained with DPNH (see Table I).

Despite all attempts to improve the efficiency of the preparations, P:O ratios higher than 1 have never been observed. This suggests that only one stage of phosphorylation may have been operating. However, in extensive studies of the two processes of electron transfer, from substrate to cytochrome *c* (in presence of KCN) and from ascorbate-cytochrome *c* to oxygen, no phosphorylation was observed in either reaction. The number of functioning phosphorylation stages is therefore indeterminate at this time. Since added cytochrome *c* increased oxygen uptake in the over-all reaction without associated phosphate uptake it is possible that in the stage of cytochrome *c* reduction, free cytochrome *c* may have been reacting out of sequence—by-passing the coupled process at this level.

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The enzymic synthesis of chitin by extracts of *Neurospora crassa*

A particulate enzyme from *Neurospora crassa*, wild strain 5297A, prepared by homogenization of frozen mycelia, in tris(hydroxymethyl)aminomethane buffer (Tris), pH 7.5, with a Ten Broeck glass tissue homogenizer, followed by collection of the material sedimented between 2000 × *g* and 140,000 × *g*, has been found to catalyze the incorporation of the N-acetylglucosamine moiety (AG) of uridine diphospho-N-acetylglucosamine (UDPAG) into an insoluble polysaccharide fraction. Neither N-acetylglucosamine-6-phosphate (AG-6-P), a mixture of N-acetylglucosamine-1-phosphate (AG-1-P) and AG-6-P, nor free AG will replace UDPAG in this reaction (Table I).

The structural similarity of the product to chitin is indicated by the following observations. (a) When radioactive polysaccharide is prepared enzymically from ^{14}C glucosamine-labelled UDPAG and then is acid-hydrolyzed, all of the radioactivity is found in the glucosamine isolated by chromatography on Dowex-50 by the procedure of GARDELL[1]. (b) AG from UDPAG is incorporated as a unit into the insoluble polysaccharide, as shown by the data in Table I where the same amount of ^{14}C was incorporated whether the UDPAG was labelled only in the glucosamine chain or only in the acetyl group. (c) When the insoluble ^{14}C-labelled polysaccharide is subjected to partial acid hydrolysis followed by removal of the acid and deionization with a mixed-bed resin (to remove any deacetylated oligosaccharides), a series of radioactive, acetylated oligosaccharides remains in solution. These substances can be separated by paper chromatography and are found to include AG, N, N'-diacetylchitobiose (using as a reference the crystalline disaccharide prepared from chitin by the method of ZILLIKEN *et al.*[2]), and higher molecular weight

TABLE I

ENZYMIC SYNTHESIS OF CHITIN FROM RADIOACTIVE UDPAG

Expt.	*^{14}C-labelled compound added to incubation mixture*	*Position of label*	*Incubation time (min)*	*Total ^{14}C in insoluble polysaccharide* (cts/min)*
A	UDPAG**, 3.8 μmoles, 76, 560 cts/min	Acetyl	0	40
	–same–	–same–	150	16,900
B	UDPAG***, 1.4 μmoles, 40, 185 cts/min	Acetyl	120	2,063
	UDPAG***, 1.9 μmoles, 50, 490 cts/min	Sugar	120	2,870
	AG-6-P***, 1.7 μmoles, 165, 480 cts/min	Acetyl	120	6
	(AG-1-P + AG-6-P)***, 1.2 μmoles, 123, 125 cts/min	Acetyl	120	20
C	UDPAG§, 1.3 μmoles, 27,040 cts/min	Acetyl	120	1,200
	AG§, 1.0 μmole, 20,000 cts/min	Acetyl	120	0

* The polysaccharide was isolated by making the reaction mixture 1 *N* in $HClO_4$. The precipitate was collected and washed four times with 2 ml portions of 0.3 *N* $HClO_4$ followed by one washing with 2 ml of water. For counting, the precipitate then was suspended in water and a suitable aliquot plated.

** Reaction mixture contained 115 mg soluble chitodextrins, 30 μmoles $MgCl_2$, 3 μmoles EDTA (versene), 150 μmoles Tris, pH 7.5. Enzyme from 4.2 g *Neurospora*. Final vol. 5.8 ml. 25° C.

*** Reaction mixture contained 2.5 mg chitodextrins, 5 mg glutathione, 50 μmoles $MgCl_2$, 15 μmoles EDTA, 250 μmoles Tris. Enzyme from 2.5 g *Neurospora*. Final vol. 5.8 ml. 25° C.

§ Reaction mixture contained 8 mg chitodextrins, 20 μmoles $MgCl_2$, 2 μmoles EDTA, 100 μmoles Tris. Enzyme from 1.5 g *Neurospora*. Final vol. 4.5 ml. 25° C. In the experiment with labelled AG, 1.5 μmoles of unlabelled UDPAG were present.

products. (d) Treatment of this mixture of N-acetylated oligosaccharides with purified chitinase yields AG which is then found to contain all of the radioactivity of the original neutral oligosaccharide mixture. The term "chitinase" is used here to refer to an enzyme activity purified 40-fold by an unpublished procedure from commercial emulsin (Nutritional Biochem. Corp.) which hydrolyzes soluble chitodextrins to AG. This enzyme activity was originally described from emulsin by ZECHMEISTER *et al.*[3].

The particulate enzyme preparation appears to contain chitin, as judged by the presence of bound glucosamine. Nevertheless, the addition of AG units from UDPAG has been found to be stimulated several-fold by the addition of high molecular weight soluble chitodextrins, prepared by the method of ZECHMEISTER AND TOTH[4]. The synthetic reaction is also markedly stimulated by AG, which, as is mentioned above, is not a participant in the reaction. The effect of the high molecular weight chitodextrins and of that of AG is distinct.

The further purification of the enzyme system from *Neurospora* and its properties are under investigation.

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