In the above equation, t, ap, hp, and h represent the time in hours, and the molar concentrations of AP, phosphohistidine and histidine resp.

A detailed report of these studies will be published elsewhere[4]. We are indebted to Professor Linderstrøm-Lang, Carlsberg Laboratory, for stimulating discussions.

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The production of β-linked glucose saccharides from cellobiose by *Chaetomium globosum*

BUSTON AND JABBAR[1] have recorded an enzymic synthesis of cellotriose by the action of enzyme preparations from *Chaetomium globosum;* they noted also that under certain circumstances a mixture of other saccharides was produced. We now report that if the organism from which the enzyme preparation was made had been grown on a medium containing cellobiose only as the source of carbon, the production of a variety of saccharides was rapid and extensive when the enzyme acted upon cellobiose.

The digest of the enzyme preparation with 5 % aqueous cellobiose was held at 30° for 9 days, and samples were removed for chromatographic examination at suitable intervals. After 2 hours, cellotriose and a second trisaccharide could be detected and the amount of these steadily increased up to 24 hours; by this time a third trisaccharide had also appeared. The first disaccharide detected was laminaribiose which was very faintly visible on the chromatogram at 4 hours; this was followed by gentiobiose at 12 hours and sophorose at 22 hours. During the next 48 hours the quantities of trisaccharides remained constant, and those of the newly-appearing disaccharides increased slightly at first and then remained constant; cellobiose decreased steadily and glucose correspondingly increased. In the later stages (after 96 hours) the trisaccharides disappeared while the disaccharides were still present, gentiobiose being predominant among these and being the only disaccharide detectable at the end of 9 days. Cellotriose in particular was more rapidly removed than any other trisaccharide, none being detectable after 96 hours.

The process thus evidently resembles that reported by BARKER, BOURNE AND STACEY[2] working with *Aspergillus niger*. These authors suggested that the three new disaccharides were formed by the coupling of glycosyl residues liberated from cellobiose with glucose molecules already present, and while this process may undoubtedly take place we are of the opinion that the disaccharides may also have been produced through the formation in the first place of trisaccharides (*e.g.*, Glu-1:6-Glu-1:4-Glu) which cleaved subsequently at the 1:4 linkage. This view is supported by the observation that at least two trisaccharides were detectable in the earliest stages of the digestion before the appearance of any disaccharide. This would imply also that addition of a glycosyl unit to cellobiose and the subsequent release of another glycosyl unit from the trisaccharide would take place at "opposite sides" of the cellobiose molecule, thus:

Glu-1:4-Glu + Glu-1:4-Glu →
Glu-1:6-Glu-1:4-Glu + Glu → Glu-1:6-Glu + Glu
or Glu-1:4-Glu-1:6-Glu + Glu → Glu + Glu-1:6-Glu

The more rapid disappearance of cellotriose might be due to the presence in the molecule of two 1:4 linkages which were apparently more readily attacked by the enzymes present than any of the other arrangements.

It may be noted that the aniline-diphenylamine-phosphate reagent of BUCHAN AND SAVAGE[3] gives very distinctive colours with the isomeric β-disaccharides of glucose. GIRI AND NIGAM[4] reported that cellobiose gave a blue colour and gentiobiose a brown; laminaribiose gives a light olive-brown and sophorose a very distinct pinkish yellow. One of the trisaccharides found here gave a distinctly pinkish yellow tone, suggesting a structural relation to sophorose.

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Enzymic activities of tumour mitochondria as evidenced by their inhibitory effect on the oxidation of DL-, D- and L- β-hydroxybutyrate by liver mitochondria

It was demonstrated in the preceding communication[1] that the oxidation of DL-β-hydroxybutyrate (BHB) by mitochondria from mouse livers prepared in isotonic sucrose, was completely inhibited in the presence of mitochondria from various tumours isolated with sucrose containing ethylenediaminetetraacetate (versene). The oxidation of octanoate was also fully suppressed under these conditions[2]. Furthermore it was found that addition of diphosphopyridine nucleotide[1] (DPN) or nicotinamide[3] (an inhibitor of DPNase) partly restored the oxidation when DL-BHB served as the substrate. No such an effect of DPN was seen, however, with octanoate.

The conclusions drawn from these and other experiments were that the tumour mitochondria possessed active splitting enzymes which destroyed DPN and adenosine triphosphate (ATP) and, consequently, abolished the fatty acid oxidative processes of the liver mitochondria. The existence of the DPNase and ATPase activities has been confirmed in direct enzymic assays[3].

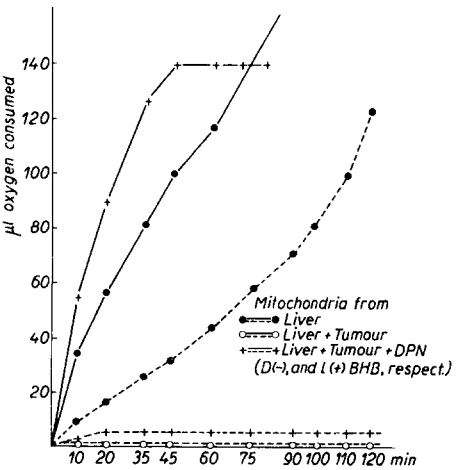

Fig. 1. The oxidation of D- and L-β-hydroxybutyrate by liver mitochondria and liver plus tumour mitochondria with and without DPN addition. Tumour: primary hepatic carcinoma rat (244 days feeding of butter yellow). Isolation of the mitochondria and incubation procedure as described[1], 12 μmoles of each stereoisomer were used. DPN was added in a final concentration of 0.001 *M*. The liver mitochondrial suspensions contained 1.75 mg nitrogen, those from the tumour 1.06 mg nitrogen.

Now, it is known from the studies of LEHNINGER AND GREVILLE[4] that the oxidation of the D(—) isomer of BHB is independent of ATP in that the free acid is oxidized, in contrast wiht the L(+) isomer which can only be oxidized as its coenzyme A (CoA) derivative and thus needs the supply of ATP for the formation of the L-BHByl-CoA bond prior to oxidation.

The oxygen consumption and the acetoacetate production which was recorded by us in the combined system of liver and tumour mitochondria oxidizing DL-BHB in the presence of DPN was usually *one half* of that shown by the liver mitochondria alone. Hence the most probable explanation[2] for this was that DPN addition resulted in the oxidation of the D-isomer by the liver mitochondria, whereas the oxidation of the L-isomer still remained blocked; this was considered as being due to the action of the tumour mitochondrial ATPase.

We have now been able to test this conclusion directly and found it to be correct, by using the pure stereoisomers of BHB. Our sincere thanks are due to Drs. GREVILLE AND LEHNINGER for their courtesy in supplying us with these samples, which were from the same stock as used by them in their own experiments[4].

Fig. 1 illustrates a typical example of the effect of DPN on the oxidation of D- and L-BHB by the liver mitochondria in the presence of mitochondria from a primary hepatic carcinoma of the rat (inbred strain R Amsterdam) induced by butter yellow feeding. In case of the D-isomer oxygen consumption is reduced to zero in the combined system of liver and tumour mitochondria. The DPNase of the latter[1,3] evidently blocks the oxidation by destroying the coenzyme of the liver D-BHB dehydrogenase, since addition of DPN restores the oxidative process. In view of the very high DPNase activity of the mitochondria from the hepatic carcinoma (complete disappearance of added DPN after 30 min contact, measured enzymically[3]) nicotinamide (0.005 *M*) was also added.

It can be seen from Fig. 1 that the oxidation of D-BHB in the combined system of liver