of 2 to 4 *M* urea increased (up to 50 % for some N-terminals) whereas concentrations of 6 to 8 *M* urea depressed (to nearly zero for some N-terminals) the extent of the autolysis of trypsin. The ε-DNP-lysine was present in all the Fractions A, B, and C. In Fraction A its amount increased linearly with the concentration of urea above 2 *M*. In Fractions B and C, however, the ε-DNP-lysine had a maximum in 2 *M* urea and the lowest value in 8 *M* urea just like the α-N-terminals; and in B the amount of ε-DNP-lysine was several times as large as in C. These overall results indicate that autolysis was maximum in 2 *M* urea and least in 8 *M* urea. In 2 *M* urea a large number of peptides were split off the protein molecule and ended up in Fractions B and C. In 8 *M* urea the unfolding of the trypsin molecule and the unmasking of the ε-lysine were most extensive.

Of great interest was the observation that the extent of autolysis paralleled the amount of flocculation of the protein in the incubation mixture at pH 7, *i.e.* flocculation was maximum in 2 *M* urea and absent in 8 *M* urea. Whether flocculation was caused by autolysis, or whether flocculated material was more easily autolysed, it is not yet known. The role of urea in these phenomena is being investigated. It was also observed that if the protein was directly suspended in 8 *M* urea small specks of material remained undissolved during the entire incubation period. Fractions B and C were then several times as large (indicating more hydrolysis) as in the case in which the protein was dissolved in 0.01 *N* HCl and dialysed prior to incubation in the 8 *M* urea. It is hoped that experiments now in progress will provide an explanation of these findings.

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A mammalian and protozoan electron-transport inhibitor in *Tetrahymena pyriformis*

An electron-transport inhibitor has been found in cell-free preparations of the protozoan *Tetrahymena pyriformis* which affects enzyme systems of *Tetrahymena* and rat liver mitochondria. The inhibitor is "activated" on mild heating or aging, it is resistant to boiling after activation but is not produced in boiled fresh preparations, and it is non-dialyzable.

Cultures were grown in 2.0 % proteose-peptone–0.2 % yeast extract medium. The cells were harvested after 3–6 days as described[1] and passed twice through a

Abbreviation: DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide.

Logeman homogenizer. Conditions of assay for the various *Tetrahymena* enzymes have also been described[2,3]. Addition of freshly-made homogenates of strain GL cells, which have extremely low succinic oxidase activity, markedly inhibits the succinic oxidase activity of homogenates of the S or W strains assayed at 30°. That a lag period of about 20 min, preceding the onset of inhibition, is abolished by heating fresh GL preparations 20 min at 50° or aging them 20 h at 0°, indicates that the inhibitor is produced or "activated" by mild heating or aging. The same, or a similar, inhibitor is found in homogenates of the S, W, E, HS, WH6, and WH14 strains. In fresh W homogenates, the inhibitor is also readily activated by heating or aging; in fresh S homogenates, it is far more resistant to activation. The inhibitor has also been demonstrated in sonates of GL cells.

The sensitivity of *Tetrahymena* succinic dehydrogenase to the GL inhibitor varies with the electron acceptor employed. With methylene blue, the dehydrogenase is inhibited to almost the same extent as the succinic oxidase system by a given amount of inhibitor, and almost complete inhibition is observed. However, with phenazine methosulfate, only 40–60 % inhibition is attained with an amount of inhibitor that completely inactivates succinic oxidase, and further large increments in the inhibitor cause only small increases in the % inhibition.

The DPNH oxidase system of S homogenates is inhibited to about the same extent as the succinic oxidase system by the GL inhibitor, as measured manometrically or spectrophotometrically. However, under conditions (incubation of S homogenate with GL inhibitor 2 h at 0°) where 83 % of the DPNH oxidase octivity is inactivated, only 9 % of the diaphorase activity (dichlorophenolindophenol reduction) is inhibited. Inhibition of β-hydroxybutyric and glutamic oxidases has also been demonstrated, but β-hydroxybutyric and glutamic dehydrogenase activities—measured manometrically with DPN, phenazine methosulphate, and cyanide—are unaffected by inhibitor levels which almost completely inactivate the oxidases. Increased amounts of inhibitor produce considerable inhibition of both dehydrogenases. The newly-described lactic oxidase of *Tetrahymena*[1], which does not involve the DPNH or succinate electron-transport chains, is not inactivated by the inhibitor.

Further studies of the GL inhibitor were directed at localizing more exactly its primary site of action in the electron-transport chain. In the protozoan, this is complicated by the total absence of cytochrome *c* oxidase activity[1] (or the presence in certain preparations of very slight activity – in press). In the hope that mammalian enzymes might be sensitive to the inhibitor and possibly furnish a better test system for determining its locus of action, rat liver was investigated. It was found that with an amount of inhibitor which inactivates 96 % of the succinic oxidase system of mitochondria, cytochrome *c* oxidase is virtually unaffected; a 5-fold increase in the inhibitor level results in 37 % inhibition of cytochrome *c* oxidase. The response of succinic dehydrogenase, assayed with phenazine methosulphate, is variable, ranging from inhibitions of 15–40 % by low levels of some GL homogenates to no effect in the presence of others. Higher levels of the inhibitor preparations greatly stimulate liver succinic dehydrogenase, the stimulation increasing with time, and this may account for the variability observed. The explanation of the enhanced activity is not yet known. Similar results for the 3 assays were obtained with liver homogenate. The inhibition of succinic oxidase is not changed when the cytochrome *c* concentration is tripled. Choline oxidase activity of rat-liver homogenate is inhibited

to about the same extent as succinic oxidase by the GL preparation. Liver DPNH oxidase activity, measured manometrically under the same conditions employed for the succinic oxidase assay, is not inhibited for at least 10 min. The greater resistance of liver DPNH oxidase to the inhibitor was also demonstrated by spectrophotometric assay (25°). 15, 30, and 60 min after incubating GL inhibitor with liver homogenate at 30°, DPNH oxidase is inhibited 0, 23 and 45 %, respectively. Similar results were obtained for DPNH-cytochrome *c* reductase, while the inhibition of succinate-cytochrome *c* reductase is almost complete after 15 min, which agrees very well with the overall succinic oxidase measurements.

If fresh homogenates (all strains studied) are boiled for 2 min, subsequent attempts to activate the inhibitor by heating or aging are unsuccessful. However, if fresh homogenates are heated at 50° for 20 min, and then boiled, or aged at 0° overnight and boiled, the inhibitor is only partially destroyed. This suggests that (a) once the inhibitor is "activated" it is remarkably heat-resistant, and (b) the activation reaction is heat-labile. Inhibition of *Tetrahymena* or liver succinic oxidase activity by a given amount of inhibitor is reduced 20–40 % by boiling the latter 20 min; 2 or 3 h of boiling or autoclaving 100 min (15 lb/in^2) destroys only slightly more inhibitor. The inhibitor is non-dialyzable in homogenates which have been aged, aged and boiled, or aged and autoclaved and is stable for at least 6 months at —13°. About 95 % of the inhibitor is localized in the particulate fraction obtained by high-speed centrifugation of heated (50°) or aged GL homogenates.

One explanation of the "activation" effect is that an enzyme, acting rapidly at 50° and more slowly at 0°, causes the formation of the inhibitor perhaps by cleavage or rearrangement of an inhibitor precursor, while boiling destroys the enzyme and thus prevents the activation process. This hypothesis is supported by the fact that the heat-activation curve of the inhibitor obtained by heating fresh homogenates from 20–80° resembles the effect of temperature on an enzyme-catalyzed reaction.

In progress are the purification and characterization of the inhibitor and a study of its relationship to the extraordinarily labile particulate electron-transport enzymes in the *Tetrahymena* homogenates[3]. Some evidence has been procured linking the latter phenomenon to the presence of the inhibitor.

More detailed information on these studies will be described elsewhere.

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