expérimentale biochimique, a d'ailleurs été critiqué (LEDERER et VILKAS[17]) et semble devoir être complètement abandonné[18,19].

[17] E. LEDERER et M. VILKAS, Vitams Horm. *24*, 409 (1966).

[18] Des expériences analogues effectuées sur des mitochondries de cœur de porc ou des particules submitochondriales phosphorylantes ont montré qu'aucune radioactivité n'est incorporée dans l'ubiquinone-10 endogène (B. FOUCHER et Y. GAUDEMER, résultats non publiés).

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Summary. During oxidative phosphorylation with cell-free extracts of *Mycobacterium phlei*, carried out in the presence of tritiated water, only negligible incorporation of tritium has been found either into endogenous MK-9(H2) or into phylloquinone added to the irradiated extract. These results rule out previously postulated mechanisms involving the enzymatic isomerization of menaquinones into quinone-methides *2*.

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A 'Dehydroascorbic Acid Reductase' Factor in Guinea-Pig Tissues

It is well established that dehydroascorbic acid reductase exists in plant tissues[1,2] and in microorganisms[3,4]. This enzyme catalyses the formation of ascorbic acid (AA) from dehydroascorbic acid (DHAA), reduced glutathione acting as the hydrogen donor. More recently, evidence has accumulated that points to the existence of a similar DHAA-reductase factor in the blood and liver of a number of species[5]. This letter reports the existence of this factor in other tissues of the guinea-pig and attempts that have been made to purify and characterize it.

Normal animals fed on a compounded pellet diet (Oxoid SGI) with a daily supplement of cabbage were killed by decapitation and the tissue to be examined was removed rapidly, homogenized in isotonic saline and the particulate fraction separated by centrifugation for 30 min at 5 °C (16,500*g*). The supernatant was dialysed for 15 h at 5 °C against 0.01 *M* phosphate buffer, pH 6.9.

'DHAA-reductase' activity was measured by determining the amount of AA formed when 4 ml of the preparation were added to a mixture of 5.75 µmoles DHAA and 16.25 µmoles reduced glutathione (GSH) in 6 ml 0.12 *M* Na_2HPO_4/KH_2PO_4 buffer, pH 6.9, and the resulting mixture incubated at 20 °C for 20 min. The reaction was stopped by the addition of 10 ml 8 % metaphosphoric acid, precipitated protein removed by centrifugation and the AA determined by the 2:6 dichlorophenol-indophenol method[6].

Results (expressed as nm moles AA formed per min per g protein) for guinea-pig preparations were: stomach 550, brain 330, adrenals 300, small intestine 240, lung 220, liver 218.

The crude preparation obtained from the stomach and small intestine was further purified. The fraction obtained between 30 % and 60 % saturation with ammonium sulphate was dissolved in the minimal amount of 0.1 *M* phosphate buffer pH 6.9 and dialysed for 15 h at 5 °C against 0.001 *M* phosphate buffer pH 6.9. The dialysate was washed through a column of Sephadex G 200 gel with 0.05 *M* phosphate buffer, pH 6.9. The active fraction was retarded by the column and it was possible to effect a 20-fold purification of the factor.

The properties of the preparation were, in general, similar to those of the liver preparation previously described[5]. The pH optimum (difficulty measurable because of the rapid increase in rate of the chemical reaction above pH 7.0) appeared to be about 6.9. Heating the preparation at 98 °C pH 6.9 for 3 min resulted in a 50 % loss of activity. Incubation with ficin for 1.5 h at 37 °C and pH 6.9 totally destroyed the activity; incubation with trypsin reduced the activity by 77 %.

Sulphydryl compounds were essential as reductants in the system; cysteine was only 20 % as effective as glutathione in this respect. $NADH_2$ was inactive as a hydrogen-donor – a finding in line with similar results obtained by other workers using bacterial preparations of the factor[7]. The factor showed 50 % greater activity towards dehydroisoascorbic acid than towards dehydroascorbic acid[5].

Azide and fluoride had no inhibitory effect at $1 \cdot 10^{-3}$ *M* concentration at pH 6.9. Iodoacetic acid, iodoacetamide and *p*-chloroacetophenone (all at $2 \cdot 10^{-4}$ *M*) produced 49 %, 56 % and 15 % inhibition respectively. Hg ions ($1 \cdot 10^{-5}$ *M*) produced 92 % inhibition.

The thermolability of the factor, the reduction in activity effected by proteolytic enzymes, the substrate specificity and the action of inhibitors are highly suggestive of the existence in animal tissues of an enzyme capable of catalysing the reduction of DHAA to AA.

The presence of a comparatively high concentration of the factor in the stomach wall is of especial significance in view of the recent reports that in the guinea-pig both DHAA and AA are absorbed from the stomach[8] and that orally administered DHAA is more effectively reduced to AA by the body than is i.p. administered DHAA[9].

Résumé. Dans des tissus de cobayes il existe une protéine capable de catalyser la réduction de l'acide déhydrascorbique en acide ascorbique. Sa distribution, sa purification, sa spécificité et son action en présence d'enzymes inhibitrices typiques ont été étudié.

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[1] B. M. CROOK, Biochem. J. *35*, 226 (1941).

[2] M. YAMAGUCHI and M. A. JOSLYN, Archs Biochem. Biophys. *38*, 451 (1952).

[3] W. B. ESSELEM and J. E. FULLER, J. Bact. *37*, 501 (1939).

[4] A. R. STEWART and P. E. SHARP, Ind. Engng Chem. analyt. Edn *17*, 373 (1945).

[5] R. E. HUGHES, Nature *203*, 1068 (1964).

[6] R. E. HUGHES, Biochem J. *64*, 203 (1956).

[7] H. TAKIGUCHI, J. Vitam. *11*, 114 (1965).

[8] R. E. HUGHES and S. C. LEWIS, Proc. Nutr. Soc. *24*, 44 (1965).

[9] P. G. DAYTON, M. M. SNELL and J. M. PEREL, J. Nutr. *88*, 338 (1966).