of the ligand to an extent that the ligand forms a benzimidazolium salt with $Cr(NCS)_6^{3-}$ instead of entering the coordination zone.

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## DEHYDROASCORBIC ACID REDUCTION IN GUINEA PIG TISSUES

DEHYDROASCORBIC ACID (DHA) is reduced nonspecifically by sulfhydryl compounds like reduced glutathione (GSH), cysteine or homocysteine. Hughes[1,2] suspected the presence of a GSH dependent DHA reductase in the guinea pig liver. However, we observed that DHA reduction in the guinea pig tissue was not enzymic but chemical and the reduction was carried out by DTNB [5-5′-dithio bis-(2-nitrobenzoic acid] reacting thiol compounds, particularly GSH. The reduction was markedly enhanced when instead of GSH, a GSH regenerating system was used.

Soluble supernatant was prepared from 20% tissue homogenates (liver, kidney and pancreas; 10% for adrenal) in isotonic Sörensen buffer, pH 7·0, by centrifuging at 105,000 × g for 1 hour.

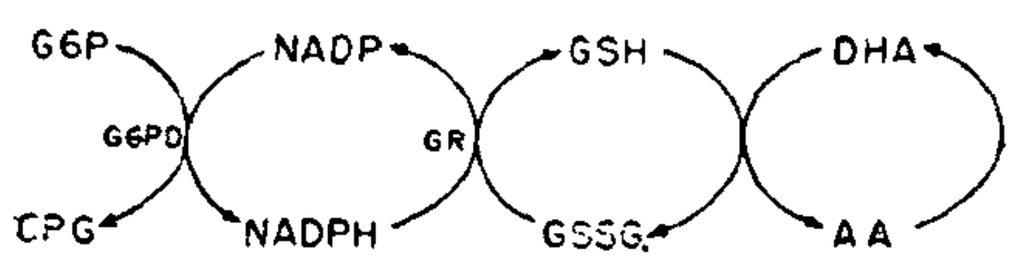
DHA was prepared freshly before use by the oxidation of AA with recrystallised *p*-benzoquinone[3]. The incubation medium contained 0·2 ml of tissue soluble supernatant, 33·4 $\mu$ moles of Sörensen buffer, pH 7·0, in a total volume of 0·9 ml. In experiments done with GSH alone, the soluble supernatant was replaced by an equivalent amount of GSH solution. The GSH regenerating system contained, in addition to the soluble supernatant and buffer, 1 $\mu$ mole G6P (glucose-6-phosphate), 0·025 $\mu$ mole NADP, 0·25 $\mu$ mole oxidised glutathione (GSSG) and 0·01 $\mu$ mole FAD. After preincubation at 37° C for 10 minutes, 0·1 ml of DHA solution (2·84 $\mu$ moles) was added and the mixture was incubated at 37° C for 5 to 30 minutes. Incubation was stopped by adding 1 ml of 10% metaphosphoric acid ($HPO_3$) solution. Ascorbic acid (AA) formed was estimated in the $HPO_3$ extract by titration with 2, 6-dichlorophenol indophenol. AA was identified by thin layer chromatography ; Rf 0·2 and 0·5 using phenol : water (5 : 1, v/v) and phenol : water : formic acid (10 : 2 : 1, v/v) respectively.

Sulfhydryl (–SH) content was estimated as GSH by the DTNB method of Beutler[4]. GR (glutathione reductase, EC 1·6·4·2) and G6PD (glucose-6-phosphate dehydrogenase, EC 1·1·1·49) were assayed by the method described in Richterich[5]. GSSG was identified by the fall of absorption at 340 nm after addition of NADPH (0·05 $\mu$ mole) and GR (0·05 unit, Sigma G 6004) to 1 ml of the incubated mixture. The incubation mixture contained 2·84 $\mu$ moles DHA, 1 $\mu$ mole GSH and 16·7 $\mu$ moles Sörensen buffer, pH 7·5; incubated for 30 min at 37° C. The products of incubation of DHA with GSH were AA and GSSG.

The DHA reducing capacity of the tissue homogenate expressed in $\mu$ moles AA formed per ml was recovered in the soluble supernatant. The reducing capacity of the soluble supernatant was dependent on the –SH content. The –SH free dialysed soluble supernatant was incapable of DHA reduction; the activity could be regained by addition of an equivalent amount of GSH. The –SH contents of different tissues expressed in terms of mg GSH/g protein were liver, 10·94, pancreas 7·41, adrenal, 4·48 and kidney, 4·04 respectively. The DHA reducing capacity of the tissues expressed in terms of $\mu$ moles of AA formed/g protein/30 minutes at 37° C was liver, 32·14, pancreas, 26·76, adrenal, 17·58 and kidney, 16·75 respectively. The soluble supernatant could be replaced by a protein free boiled extract of the soluble supernatant (10 min. at 98° C) or by an equivalent amount of GSH. When GSH was replaced by a GSH regenerating system consisting of soluble supernatant, G6P, NADP, GSSG and FAD, the rate of DHA reduction was markedly enhanced (Fig. 1). In this system, the rate of DHA reduction was dependent on both the G6PD and GR activities of the soluble supernatant. The G6PD activities of the soluble supernatant of liver, pancreas, kidney and adrenal were 16·35, 66·98, 38·42 and 100·50 I.U./g protein respectively; the corresponding GR activities were 72·66, 130·98, 116·12 and 44·65 I.U./g protein respectively. The results given in Fig. 1 indicate that in the guinea pig tissues, DHA reduction is coupled with the GSH regenerating system as shown in Scheme 1. The oxidation of AA to DHA takes place probably in the mitochondria via cyt $c$[6]. In the GSH regenerating system, the tissue soluble supernatant could be replaced by an equivalent amount of G6PD (Sigma G 1878) and GR (Sigma G 6004). Dehydroepiandrosterone ($4 \times 10^{-5}$ M), which inhibited G6PD activity of tissue soluble supernatant by 80%, inhibited the DHA reduction by about 80%.

The validity of Scheme I was also supported by the fact that in the –SH free liver soluble supernatant the rate of reduction of DHA was dependent on the concentrations of GSSG and NADP. In comparison with

that obtained with 0·25 μ mole GSSG (Fig. 1), there was a decrease in DHA reduction by 47%, 73% and 87% respectively with 0·1 μ mole, 0·05 μ mole and 0·025 μ mole GSSG. Similarly, in comparison with that obtained with 0·025 μ mole NADP (Fig. 1), the DHA reduction was less by 30%, 67% and 83% respectively with 0·01 μ mole, 0·005 μ mole and 0·0025 μ mole NADP. There was no reduction of DHA in the absence of GSSG or NADP.




SCHEME I. Schematic representation of DHA reduction in the guinea pig tissues.

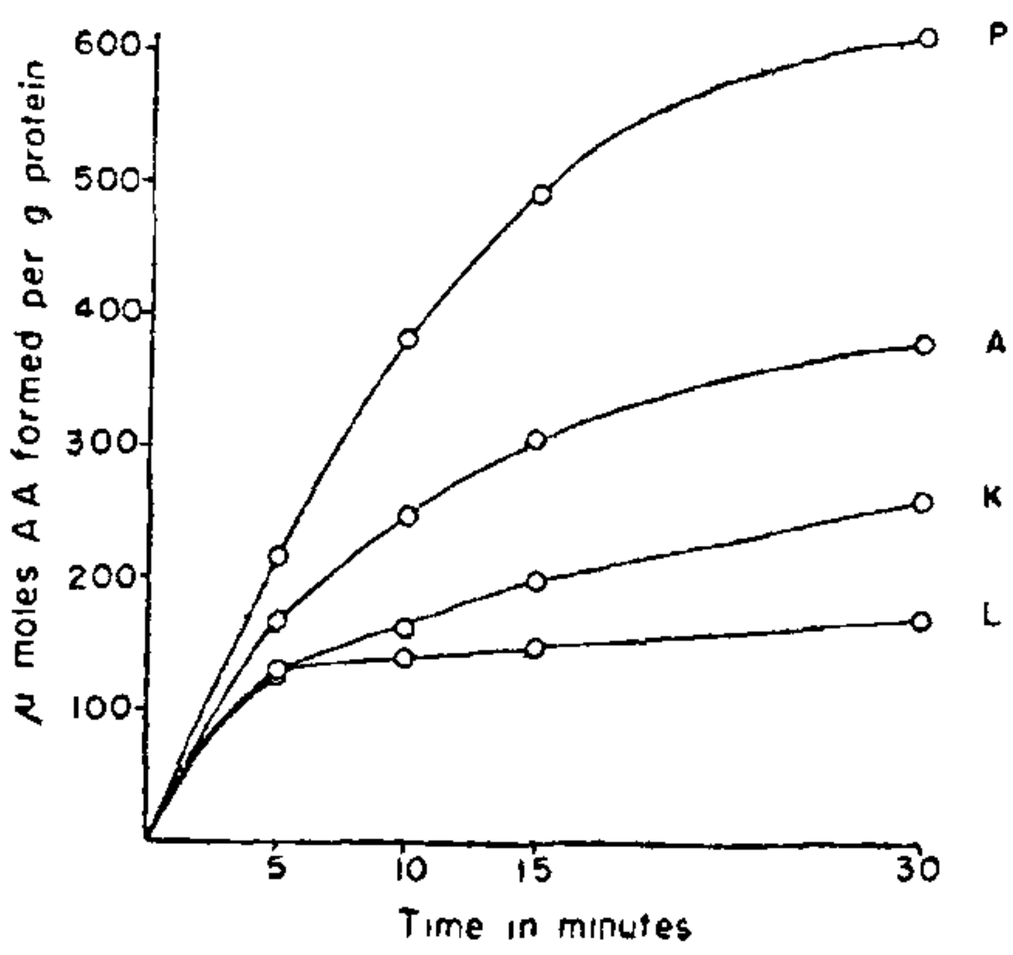



FIG. 1. DHA reduction in the guinea pig tissue soluble supernatant using GSH regenerating system. L, liver; K, kidney; P, pancreas and A, adrenal. The experimental procedure is described in the text

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## REGENERATION OF WHOLE PLANTS BY EMBRYOGENESIS FROM CELL SUSPENSION CULTURES OF SANDALWOOD

SANDALWOOD (*Santalum album* L.) is one of the many forest trees difficult to propagate vegetatively by the usual methods. There is a growing demand for sandalwood and its products in the International market. However, there is a considerable reduction in the production from 1974 onwards. Several factors have contributed for the low production[1]. One of the main problems is the spike disease caused by mycoplasma. Plants from 3 years onwards are susceptible to the disease resulting in death. To meet the growing demand, trees of improved quality and disease resistance are needed. Present-day tree improvement programmes are not adequate to meet the growing demand. In recent years, the technique of plant tissue culture has been hailed as the answer to these problems. It can, in principle, produce untold numbers of plants all genetically identical. Until recently application of tissue and cell cultures for propagation is confined mostly to herbaceous plants[2]. The success in tree tissue cultures is comparatively limited. The most pressing problem currently confronting the forest researchers is, to develop an economically efficient technique of propagation from callus and cell cultures. The technique must be suitable to be applied to both hardwood and softwood trees, that are difficult to propagate vegetatively by the usual methods of grafting, or by the rooting of branch cuttings. Regeneration in the form of embryos or plantlets is reported for other forest trees[3–5]. However, except for a few, the frequencies are low and not yet of any commercial interest. Most of these studies are confined to seed embryos or seedling explants. We have earlier reported[6] the somatic embryogenesis in sandalwood from mature trees. For large scale commercial application, the only practical method in the long run is embryogenesis in suspension cultures. We report here embryogenesis in suspension cultures leading to plantlet formation.

Sandalwood shoot callus was isolated as previously reported[6]. Murashige and Skoog's[7] (MS) and White's[8] (WM) media were used as basal media. Basal media were supplemented with auxins, cytokinins and gibberellic acid in different experiments either singly or in combinations. Suspension cultures were maintained in 250 ml conical or nipple flasks at pH 5·2. Conical