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## Coenzyme $Q_{10}$ and Succinate-Tetrazolium Reductase Activity of Proliferative Lesions of Liver.* (25848)

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Histochemical studies of the succinic dehydrogenase system using tetrazolium salt reduction technics have shown a decrease of succinate-tetrazolium reductase activity in a number of benign and malignant proliferative lesions(1,2,3). Recent work employing the tetrazolium salt, 2 - (p - iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), has indicated that a quinone, presumably coenzyme $Q_{10}$ ($CoQ_{10}$), serves as an intermediate electron transport agent in the succinate-INT reductase reaction and that content of quinone in normal tissues is below that required for maximum rate of activity of this reaction(4). In the present investigation quantitative histochemical determinations of succinate-INT reductase activity of sections of normal liver, regenerating liver and the Novikoff hepatoma have been carried out in presence of added $CoQ_{10}$ and also in presence of a second quinone, 2-methyl-1,4-naphthoquinone (menadione). These experiments were performed to determine the individual roles of the primary dehydrogenase and quinone in decreases in reductase activity which have been observed. Parallel studies have also been carried out on a second reductase system, alpha-glycerophosphate-INT reductase, in which a quinone electron transport agent takes part in a manner similar to that of the succinate-INT reductase system(4).

*Materials and methods.* Quantitative procedures have been described(4). Frozen and dried sections 16 $\mu$ thick, with a dry weight of approximately 0.5 mg were incubated in a reaction mixture containing sodium succinate or sodium alpha-glycerophosphate, 0.05 M; NaCl, 0.11 M; KCl, 0.003 M; $MgSO_4$, 0.001 M; $Na_2HPO_4$, 0.03 M; acetone 5%; and INT, 0.4 mg/ml; pH was adjusted to 7.4. Sections were incubated at 37° rather than 28° as in the former investigation because of inclusion in this study of tissues with very low activity. At the higher temperature the enhancing effect of the quinones is relatively less compared to total activity of the section than at the lower temperature. An incubation period of 15 minutes was used for determination of succinate-INT reductase activity and 30 minutes for alpha-glycerophosphate-INT reductase activity. As in the earlier work, addition of $CoQ_{10}$ is made by coating the compound on the coverslip and placing the tissue section in direct contact with the dried material. A considerably greater enhancement of enzyme activity has been found with this method than by placing the quinone directly into reaction mixture. For this procedure, 0.02 ml of a solution of $CoQ_{10}$, 1 mg/ml, in equal volumes of ethyl ether and acetone was placed on a 22 mm square coverslip and the solvent allowed to evaporate at room tempera-

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TABLE I. Effects of Quinones on Succinate-INT Reductase and Alpha-Glycerophosphate-INT Reductase Activities of Normal and Rapidly Proliferating Hepatic Cells.

| | | Quinone additions | | | Quinone additions | |
|---|---|---|---|---|---|---|
| | | None | $CoQ_{10}$ | Menadione | $CoQ_{10}$ | Menadione |
| | | Enzyme activity* | | | Increased enzyme activity | |
| Enzyme system | Type of hepatic tissue | Units† | Units† | Units† | %‡ | %‡ |
| Succinate-INT reductase | Normal | 190 ± 13 | 283 ± 28 | 312 ± 26 | 49 | 64 |
| | 24 hr regenerating | 127 ± 20 | 196 ± 20 | 283 ± 29 | 54 | 123 |
| | 48 " " | 91 ± 3 | 159 ± 19 | 243 ± 19 | 75 | 167 |
| | Hepatoma | 54 ± 10 | 221 ± 14 | 143 ± 7 | 310 | 165 |
| Alpha-glycerophosphate-INT reductase | Normal | 25 ± 5 | 42 ± 11 | 65 ± 12 | 68 | 160 |
| | 24 hr regenerating | 14 ± 3 | 17 ± 4 | 60 ± 17 | 21 | 329 |
| | 48 " " | 15 ± 4 | 19 ± 4 | 61 ± 8 | 27 | 307 |
| | Hepatoma | 52 ± 6 | 133 ± 18 | 210 ± 34 | 156 | 304 |

* Mean ± stand. dev. Each value represents 8 animals.
† 1000 × △O.D. at 490 mμ/mg dry wt/min.
‡ 100 × (activity with quinone addition − activity without addition)/activity without addition.

ture. Menadione was added directly to the reaction mixture and was present at a concentration of 0.2 mg/ml. Female Sprague-Dawley rats 3 to 4 months of age obtained from the Holtzman Co. were used. In the hepatic regeneration experiments approximately two-thirds of the liver was excised(5). The Novikoff hepatoma was grown intraperitoneally as a solid tumor(6). Tumors 4 or 5 days following transplantation were used for the quantitative work.

*Results.* The results are summarized in Table I. Succinate-INT reductase activity of regenerating liver and the Novikoff hepatoma is considerably less than that of the normal liver. Succinate-INT reductase of normal liver and the proliferating lesions of the liver are not saturated with respect to quinone as evidenced by the fact that in these tissues, activity of this system is enhanced by addition of either $CoQ_{10}$ or menadione to the reaction. In regenerating liver, degree of enhancement obtained with $CoQ_{10}$ is slightly greater than in normal liver. Addition of menadione causes a considerably greater enhancement of reductase activity than that found in the normal liver, with the result that activity of regenerating liver approaches that of normal liver when both are incubated in presence of this quinone. In the Novikoff hepatoma, $CoQ_{10}$ enhances succinic-INT reductase activity to a very marked degree so that addition of this quinone brings activity of the hepatoma close to that of normal liver incubated under the same conditions. Menadione is less effective in this regard.

The results obtained with alpha-glycerophosphate-INT reductase system in certain respects parallel those of succinate-INT reductase. Thus, in the regenerating liver, activities of both reductases are profoundly enhanced by menadione whereas $CoQ_{10}$ is considerably less effective. An additional parallelism is found in the hepatoma. In this instance, activities of both of the reductases are markedly enhanced by $CoQ_{10}$. A major point of difference is that alpha-glycerophosphate-INT reductase activity of the hepatoma is greater than that of the normal liver, in sharp contrast to the situation existing with succinate-INT reductase.

*Discussion.* The frequent occurrence of low succinate-tetrazolium reductase activity in rapidly proliferating tissues suggests that a decrease in activity of this system is part of a metabolic alteration associated with rapid cellular proliferation in at least certain anatomic structures. The results of the present work indicate that in liver a considerable proportion of this decrease in activity is due to an alteration in the quinone component of the system. Recently reported data indicate that $CoQ_{10}$ is an intermediate electron transport agent between succinic dehydrogenase and the cytochrome chain(7). Accordingly, the findings presented here suggest the possibility that in

proliferating cells one or more mechanisms may exist which result in succinic dehydrogenase and also alpha-glycerophosphate dehydrogenase becoming dissociated from $CoQ_{10}$ and the cytochrome electron transport chain.

The nature of the alteration of quinone-dehydrogenase relationship which occurs in the regenerating liver and hepatoma appear to differ. In considering the pattern of response of the reductases of regenerating liver to the 2 quinones, account should be taken of the fact that regenerating liver is a sharply self-limited proliferative process occurring in an adult tissue. Accordingly, an attractive hypothesis for explaining the effects of the 2 quinones on this tissue is that the proliferative mechanism is related to a reversible dissociation of the 2 dehydrogenases from the electron transport system by virtue of some effect on $CoQ_{10}$. Under these conditions it might be more difficult for $CoQ_{10}$ added *in vitro* to couple with the dehydrogenase than it would be for an unnatural quinone such as menadione. In the hepatoma this type of postulated reversible mechanism would presumably be lacking. The pronounced enhancing effect of $CoQ_{10}$ on the 2 reductases of the hepatoma suggest that in this tissue there simply is a lack of available quinone which is most efficiently supplied *in vitro* by addition of the naturally occurring quinone.

*Conclusion.* Quantitative histochemical studies of sections of normal liver, regenerating liver and Novikoff hepatoma have shown that the succinate-INT and alpha-glycerophosphate-INT reductase systems of these tissues are not saturated with respect to quinone. Compared to normal liver, degree of unsaturation is considerably greater in regenerating liver and hepatoma and largely accounts for those low reductase activities which are observed in these 2 tissues. Response of sections of regenerating liver and hepatoma to $CoQ_{10}$ and menadione differs. $CoQ_{10}$ is very effective in hepatoma. In regenerating liver $CoQ_{10}$ is relatively ineffective whereas enhancement of reductase activity brought about by menadione is profound.

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## Dietary Fats and Effects of Internal Radiation by $P^{32}$.* (25849)

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Several groups of workers reported that addition of corn oil, cottonseed oil, or methyl linoleate to a fat-free diet significantly decreased mortality of mice(1) or rats(2-6) subjected to total body irradiation. On the other hand, our previous data indicated that mice injected with single midlethal dose of $P^{32}$ exhibited greater susceptibility to the isotope when fat content of diet was raised from 5% to 30% level(7). We have now compared effects of $P^{32}$ on mice fed a fat-free diet with those observed in mice fed diets with high content of either a saturated fat (coconut oil), or an unsaturated fat (corn oil). Mortality was lowest in groups on fat-free diet. However, mice fed corn oil showed a better survival than those on coconut oil diet.

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