Converting Enzyme Content of Different Tissues of the Rat

The major role played by the lungs as a site of conversion of circulating angiotensin I to angiotensin II has been demonstrated by VANE et al.[1,2] and confirmed by others[3,4]. The fact that such organs as liver and kidney seem to play a negligible part as sources of the converting enzyme, raises the question whether this enzyme is specifically produced by the lungs. This led us to investigate the converting enzyme content of different tissues of the rat. We used a chemical assay[5], in which the conversion of the synthetic substrate, Z-phenylalanyl-histidyl-leucine[6] to Z-phenylalanine and histidylleucine, is used as an index of enzyme activity. This conversion has been shown to be similar to that of angiotensin I to angiotensin II[6].

Male rats weighing from 180–200 g were anaesthetized with ether and decapitated. The organs to be analyzed were removed and homogenized in a glass Potter homogenizer (1 part by weight with 8 parts by volume of 0.25 *M* sucrose). Intestine was homogenized with a blade homogenize. The homogenates were frozen and thawed 6 times. In a number of experiments, the blood contained in organs was removed prior to homogenization by perfusion with a Ringer solution containing 1% heparin. Otherwise, the organs were thoroughly washed with this solution. The heart and intestine were opened with scissors before washing.

To assay the enzyme, 20 µl of homogenate supernatant were incubated 15 or 30 min at 37 °C with 3 ml of 0.05 *M* phosphate-borate buffer (pH 8.0, containing 1% NaCl) and 50 µl of substrate solution (2 mg/ml in methanol). The reaction was stopped by addition of 0.4 ml of 2 *N* NaOH, and histidylleucine was assayed fluorimetrically[5]. The protein concentration of the samples was assayed with the biuret reaction.

Concentration of the converting enzyme in different tissues of the rat

Tissue	Protein (mg/ml)	Converting enzyme (mU/mg protein) Individual samples	Average
Lung	7.9[a]	30	37.2
	6.5[a]	49	
	6.3[a]	60	
	17.6	24	
	14.6	23	
Liver	25.3[a]	0.23	0.23
	22.1[a]	0.23	
Kidney	18.0[a]	0.40	0.63
	17.6[a]	0.80	
	22.0	0.70	
Heart	24.6	0.10	0.15
	20.3	0.20	
Brain	24.2	1.10	1.47
	23.8	1.60	
	21.6	1.70	
Small intestine	13.9	4.30	4.67
	9.9	5.50	
	14.1	4.20	
Stomach	12.9	2.66	2.18
	15.8	1.70	
Muscle	22.9	1.10	0.71
	16.9	0.63	
	22.7	0.40	
Serum	54.3	0.74	1.03
	57.4	0.90	
	54.3	0.82	
	50.2	1.08	
	64.7	0.72	
	57.4	0.95	
	60.5	1.22	
	57.4	1.78	

[a] Perfused organ.

The results (Table) show that the lung contains far more converting enzyme than any other tissue investigated: its content exceeds that of liver by more than 100 times, and that of small intestine, which has the highest content after lung, by about 8 times. These findings are in good agreement with the work of VANE et al.[1,2] who reported that angiotensin I is converted to angiotensin II in the vascular bed of the lung. Few examples of lung-specific enzymes are known so far, and it would be interesting to know whether pulmonary diseases occur in which the converting enzyme level of blood is modified[7].

Résumé. On a dosé l'enzyme de conversion dans différents tissus du rat au moyen d'une méthode chimique mesurant la libération d'histidyl-leucine à partir du substrat synthétique Z-phe-his-leu. Le poumon contient au moins 8 fois plus d'enzyme que tous les autres tissus étudiés. Il en contient notamment plus de 100 fois plus que le foie.

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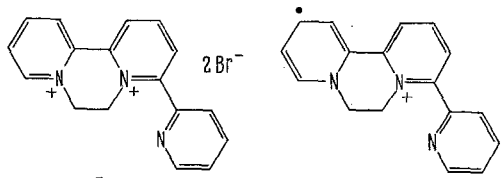
One Electron Transfer Properties and Phytotoxicity of a Diquaternary Salt from 2,2′:6′,2″-Terpyridine

The bipyridylium salts, diquat and paraquat, are established herbicides[1] but the mode of their biological action is not yet completely clear[2], although much evidence has been obtained[3] to support the view[4,5] that it is associated with their ability to be reduced at a potential (E_o) of about −0.40 volts to free radical cations by a one-electron transfer which is completely reversed by air. A few reports, however, indicate[3] that the size of the molecule is important in determining whether a compound possesses herbicidal properties. In particular, certain diquaternary salts of 4,4′-bipyridyls substituted with 2 aromatic rings are inactive[6], although they meet the requirements thought to be necessary for activity. In view of this report, we have investigated a diquaternary salt of 2,2′:6′,2″-terpyridine which can be considered to be a bipyridyl substituted with one aromatic ring.

2,2′:6′,2″-Terpyridine reacted with boiling ethylene dibromide to give 6,7-dihydro-4-(2-pyridyl)-dipyrido[1,2-

$a:2',1'$-c]pyrazinium dibromide (I), which crystallized from aqueous ethanol as yellow-green needles of the monohydrate, mp 290° (dec.) (Found: C, 46.4; H, 3.7; N, 9.4; $C_{17}H_{15}Br_2N_3 \cdot H_2O$ requires C, 46.5; H, 3.9; N, 9.6%). The NMR-spectrum in D_2O consisted of a singlet at $\delta = 5.3$ and a complex multiplet in the range $\delta = 7.7$–9.3 ppm with an area ratio of 4:11. The UV-spectrum showed maxima at λ 275 (log ε 3.80) and 326 nm (4.32).



I II

An aqueous solution of (I) on treatment with zinc dust developed immediately an intense green coloration due to the corresponding radical cation (II). The presence of a high concentration of a stable radical was confirmed by the fact that the intensely coloured solution gave essentially no NMR-spectrum. When the reducing agent was removed and the solution was shaken in air the deep colour discharged. The NMR-spectrum obtained then was identical with that of the original salt indicating, like diquat and paraquat, that the one electron transfer is essentially completely reversible. On polarographic examination in the pH range 1.5 to 8.2, (I) gave a typical symmetrical one-electron reduction wave with a half-wave potential (E_0) of -0.32 volts independent of pH. A second reduction wave which approximated to the uptake of one electron at a potential of -0.72 volts was also present above about pH 6. At lower pH the second wave was less clearly defined and the half-wave potential was pH dependent. This behaviour is reminiscent of that noted recently[7,8] with a number of similar diquaternary salts and is presumably associated with protonation of the radical cation at low pH.

In post-emergent herbicidal tests[3] in the greenhouse, the salt (I) gave a complete kill of 6 plant species when applied at a rate equivalent to 7 lb/acre. In tests at lower application rates on mixed grass flora it was found to be about one-sixth as active as diquat. These results confirm that compactness of the molecule is necessary for outstanding phytotoxic properties. The considerable herbicidal activity obtained from (I), however, suggests that difficulty in reaching the site of biological action of the bipyridylium herbicides is responsible for its reduced activity rather than an inability to participate in the toxic mechanism, although the higher reduction potential of (I) which may not be low enough to exert the fullest effect on the biochemical electron transfer processes with which the bipyridylium herbicides are thought to interfere may be a contributing factor. The salt (I) with its redox potential of -0.32 volts provides a useful extension to the range of completely reversible redox indicators of the viologen type which have hitherto been confined[4] to potentials below -0.35 volts. KOK, RURAINSKI and OWENS[9] have also recently reported a salt with a redox potential of -0.32 volts.

Zusammenfassung. 2,2′:6′,2″-Terpyridin gibt mit Aethylendibromid ein diquartäres Salz, das durch Reduktion mit Zink eine Radikalkation liefert. Es handelt sich um ein Redox-System und das Salz besitzt herbizide Wirksamkeit.

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Rat Hepatocyte Peroxisomes: Ultrastructural Alterations Following Cessation of Chronic Dietary Clofibrate Administration

The purpose of this note is to present our observations of previously undescribed ultrastructural alterations in the matrix of hepatocyte peroxisomes in animals studied 3 weeks following an initial 6-week chronic dietary Clofibrate (CPIB) administration.

Recent studies in our laboratories concerning the response of the rat liver to ethyl-chlorophenoxyisobutyrate (Clofibrate – CPIB) confirm previous reports of hepatomegaly characterized ultrastructurally by a marked increase in cytoplasmic hepatocyte peroxisomes (microbodies)[1–5]. Our findings were noted after 6 and 12 weeks dietary administration of CPIB (0.3% diet) to Sprague-Dawley rats.

In addition, studies were performed to define the ultrastructural features of the hepatocyte 3 and 6 weeks following an initial period of 6 weeks dietary administration of CPIB. The results of these studies indicate reversibility to normal considering absolute and relative liver weight relationships, light microscopy and ultrastructural features at both post-treatment sampling times. No ultrastructural evidence of adverse residual cell damage was noted.

Abbreviated protocol of experiment. Eight 150 g Sprague-Dawley (Charles River) rats were assigned to each group. Body weight, food consumption and symptomatology were recorded weekly. Liver specimens were collected from each of the 4 major lobes for both light microscopy (Formalin – H. & E. and Oil Red O) and electron microscopy (glutaraldehyde and osmium fixation – epon araldite embedment). 4 treatment regimens were per-

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