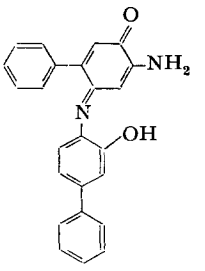
The enzymic oxidation of o-aminophenols*

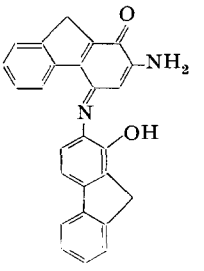
The binding of a variety of chemical carcinogens or their metabolites to cellular proteins is currently considered a causative factor in the induction of neoplasms[1]. In the majority of instances the reactive metabolites have not been identified. In the case of carcinogenic aromatic amines the suggestion has been made that the binding reaction involves, as intermediates, quinone imines which may arise from oxidation of aminophenols[2]. The latter compounds are recognized as metabolic products of aromatic amines[3]. The present report provides evidence heretofore not available that *o*-aminophenols can be oxidized *directly* by the cytochrome *c*–cytochrome oxidase system. In contrast, tyrosinase apparently does not act directly on the *o*-aminophenol, 3-hydroxykynurenine, the oxidation being dependent on the presence of 3,4-dihydroxyphenylalanine (DOPA)[4]. In this system, the *o*-quinone formed from DOPA by the action of tyrosinase oxidizes the aminophenol. In both systems, however, the initial oxidation products are very likely *o*-quinone imines.

Addition of equimolar amounts of oxidized cytochrome *c* to 1-hydroxy-2-aminofluorene (I) in 0.1 *M* phosphate buffer, pH 7.4, resulted in the instantaneous reduction of cytochrome *c*, as shown by the appearance of the absorption bands of reduced cytochrome *c*. When mitochondria from rat-kidney cortex were incubated in a buffered medium with I and catalytic amounts of cytochrome *c*, oxidation of the aminofluorenol was complete in 60 min, as indicated by the disappearance of the aminofluorenol band at 280 mμ. No oxidation was detectable by spectrophotometric measurements in the absence of cytochrome *c*. Enzymic oxidation of *o*-aminophenol (II), 3-hydroxy-4-aminobiphenyl (III), and I by cytochrome *c* and a soluble preparation of cytochrome oxidase[5] gave oxidation products which, when extracted into suitable organic solvents, exhibited characteristic visible absorption spectra. The oxidation product of II gave a spectrum with twin peaks at 422 and 437 mμ. This spectrum proved to be identical with that of 2-amino-3H-isophenoxazin-3-one[6]**, m.p. 255–256°, λ_{max} 422, 437 mμ (ε = 24,400; 25,000) in ethanol, which was prepared by treatment of II with activated lead dioxide. Ascending paper chromatography on Whatman No. 1 paper using as solvent the lower phase of butanol–ethanol–water (5:2:10) gave identical R_F values (0.40) for both the enzymic and the synthetic oxidation products. Cytochrome *c*–cytochrome oxidase oxidized III to a product which absorbed maximally at 413 mμ. The identical spectrum was exhibited by 1,7-diphenyl-4-amino-3H-isophenoxazin-3-one (IV). This new compound was synthesized by ferricyanide oxidation of III in 0.1 *M* phosphate buffer, pH 7.4; yellow-orange needles from ethanol–water, m.p. 194–196° decomp. Calculated for $C_{24}H_{16}N_2O_2$: C, 79.1; H, 4.43; N, 7.69; molecular wt. 364; for $C_{24}H_{16}N_2O_2 \cdot H_2O$: C, 75.3; H, 4.74; N, 7.34. Found: C, 75.3; H, 4.85; N, 7.62. After chromatography on acid-washed, activated alumina and recrystallization from ethyl acetate–light petroleum, found: C, 78.6; H. 4.87; N, 7.47; molecular wt. (cryoscopic, benzene) 354; λ_{max} 413 mμ (ε = 21,100) in chloroform. Paper chromatography in butanol–acetic acid–water (1:1:7) again confirmed the identity of the synthetic and enzymic products (R_F 0.81, 0.80). Lead dioxide oxidation of III did not give IV, but a blue-violet compound V which was contaminated with lead, λ_{max} 555 mμ in ethyl acetate. Attempts to purify V by column chromatography on alumina converted it to a large extent to IV. IV was also obtained when V was warmed in 0.1 *M* phosphate buffer, pH 7.4.

I, whether oxidized enzymically or by lead dioxide, gave a product absorbing maximally at 585 mμ. On the basis of molecular-weight determinations and the similarity of its visible spectrum with that of V and that of the air oxidation product of 2-amino-1-naphthol[7], structure VI has been tentatively assigned to this product.



V



VI

The products formed in these enzymic and/or chemical oxidations of *o*-aminophenols can readily be accounted for by a mechanism involving *o*-quinone imine intermediate[8]. Since the enzymic oxidations in this work were carried out with a soluble system, the question may be raised whether the oxidation products isolated under these conditions indeed represent end products of the cellular

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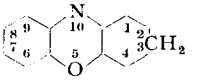
** To obviate ambiguity in the nomenclature of these compounds containing the isophenoxazine ring structure, the current Chemical Abstract system of nomenclature and numbering is used; thus, 3H-isophenoxazine =



metabolism of *o*-aminophenols. Further studies are required to settle this point. If *o*-aminophenols are further oxidized within the cell through the mechanism suggested by the present experiments, an appreciable fraction of the generated *o*-quinone imines might be expected to bind free functional groups of protein side chains in a manner analogous to *o*-quinones[9].

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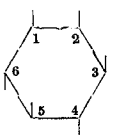
Metabolism of 2-^{14}C-*myo*-inositol in the rat*

Several workers have demonstrated the conversion of *myo*-inositol to glucose in the rat[1,2,3,4], and a recent report has indicated that kidney extracts are capable of converting inositol** to DL-glucuronic acid[5]. The availability, in our laboratory, of 2-^{14}C-*myo*-inositol*** has made it possible further to investigate the metabolic pathways involved.



myo-Inositol

"Physiological" doses of 2-^{14}C-inositol (0.7 to 3 mg, 0.86 to 3.7·10^6 counts/min, < 1/10 of the normal daily intake) were injected intraperitoneally into well-fed, adult male rats. Useful activity (1.5 to 3 % of the dose) could be recovered from the liver glycogen 8 to 12 h after the injection. The glycogen, isolated from liver and muscle by the method of STETTEN AND BOXER[6], was hydrolyzed to glucose, and this was degraded carbon by carbon *via Leuconostoc* fermentation[7]. The results are shown in Table I.

TABLE I

^{14}C DISTRIBUTION IN GLUCOSE FROM GLYCOGEN AFTER INJECTION OF 2-^{14}C-*myo*-INOSITOL

All samples were counted as barium carbonate after plating on copper planchets. The figures are corrected for self-absorption.

	Counts/min/mmole/C		
	Expt. 1		*Expt. 2*
	Liver	*Muscle*	*Liver*
Whole glucose	429	210	2457
Carbon 1	1285	633	6800
Carbon 2	89	95	691
Carbon 3	62	34	612
Carbon 4	84	62	590
Carbon 5	87	83	818
Carbon 6	1339	478	5262

In all cases, whether the glucose was derived from liver or from muscle glycogen, over 80 % of the radioactivity was found in positions 1 and 6, which were about equally labeled. These results parallel those of POSTERNAK *et al.*[2], who found that 2-^{2}H-*myo*-inositol was converted to 6-^{2}H-D-glucose by a phlorizinized rat. The appearance of label in the 1-position of the glucose is, however, a new finding. No deuterium was found there by the Swiss workers; presumably carbon destined for this position lost its deuterium in the conversion process.

The present data are consistent with the hypothesis that 5-^{14}C-D-glucuronic acid, formed by cleavage of the inositol between carbons 1 and 6, is an intermediate in the conversion to glucose. According to the scheme of BURNS AND KANFER[8], 5-^{14}C-D-glucuronic acid would be converted to 5-^{14}C-D-xylulose. This, after phosphorylation, would yield 3-^{14}C-3-phospho-D-glyceraldehyde, which would account for the label in position 6 of the glucose, and for a good deal of that in position 1. The exact amount would depend on the degree of isomerization of the triose phosphate. If L-glucuronic acid is

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** Whenever the term "inositol" is used, the *myo*-isomer is implied.
*** The synthesis will be described in a later publication.