cholamine biosynthesis induced by potassium appears to be a more potent effect than that induced by nerve stimulation. Thus, nerve stimulation for 1 h periods produces variable increases in NE formation which seldom exceed 100%[2,4]. On the other hand, the increase induced by potassium (40%) is always greater than 100%. However, preliminary experiments do indicate that like the acceleration of NE biosynthesis induced by nerve stimulation, the acceleration induced by potassium can also be blocked by the presence of NE ($10^{-6}M$) in the medium. We are currently investigating the mechanism of this potassium-induced acceleration of catecholamine biosynthesis.

Zusammenfassung. Kalium erhöht die Bildung von markiertem Noradrenalin aus markiertem Tyrosin im isolierten Vas deferens. Es wird angenommen, dass dieser Effekt auf eine Steigerung der Tyrosin-Hydroxylase zurückzuführen ist.

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Studies on the Biochemical Characterization of Human Ceruloplasmin

The pathological picture of Wilson's disease has been attributed to the abnormal deposition of copper in liver, kidney and brain, along with a decrease in serum ceruloplasmin[1]. That no direct relationship, however, has been observed between serum ceruloplasmin concentration and the severity of the disease[2-4], has led us to a hypothesis for the independent role of copper and ceruloplasmin in the possible pathogenesis of Wilson's disease.

Materials and methods. The ceruloplasmin was obtained from serum by adsorption onto DEAE-Sephadex and by ammonium sulfate precipitation. $^1/_{10}$% ceruloplasmin in sodium acetate buffer (0.1 *M*, pH 5.5) was used for the analytical ultracentrifuge at 59,780 rpm. Disc gel electrophoresis was performed at pH 8.9 for 45 min to 1 h at 4 mamp/sample with a 7.5% cross-linked polyacrylamide gel.

Oxidase activity was detected essentially by the method of Owen and Smith[5]. Subunits were determined after treatment of the protein in 9 *M* urea with 2-mercaptoethanol and iodoacetamide[6] followed by chromatography on DEAE-Sephadex. Peptide maps were prepared of tryptic digests by electrophoresis (70 V/cm; pyridine acetate, pH 5.5; 45 min) in one dimension and chromatography (butanol:acetic acid:water – 200:30:75; 16–18 h) at right-angles. Mitochondrial respiration in the presence of ceruloplasmin was measured with a YSI Oxygen Monitor (Yellow Springs Instrument Co., Ohio) using a rat-liver preparation as noted in the footnote to the Table.

Results and discussion. Preliminary chromatographic separation of the subunit reaction mixture of bovine ceruloplasmin revealed a single major peak while that of the human protein showed 2 principal peaks. In disc gel electrophoresis as well as with the cellulose acetate procedure, normal ceruloplasmin migrates further towards the anode than does the Wilson protein. The basic nature of

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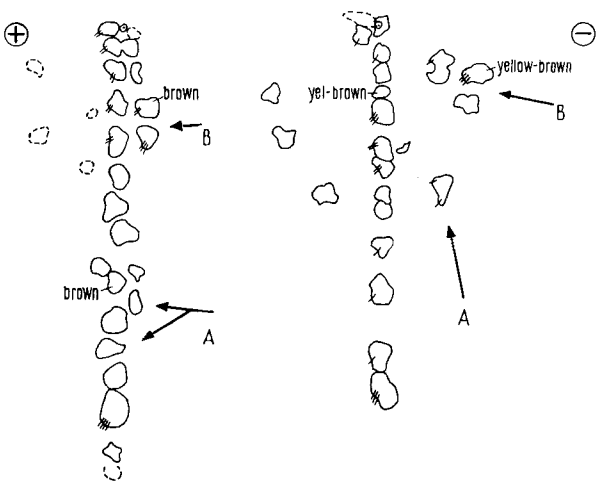
Effect of ceruloplasmin on mitochondrial oxygen consumption

Incubation medium[a]	O_2 consumption, µl/h/mg protein	Inhibition (%)
No Cp	190	–
200γ Bovine Cp	170	10.5
200γ Normal Cp	168	11.6
200γ Wilson's Cp	27	85.8
80γ Wilson's Cp	27	85.8
60γ Wilson's Cp	67	64.7
40γ Wilson's Cp	80	57.9
20γ Wilson's Cp	150	21.1
0.2 m*M* Cu^{+1}	190	0
0.2 m*M* Cu^{+2}	57	70.0

[a] Mitochondria were prepared from rat liver and suspended in buffer so that 0.2 ml of preparation had 0.672 mg protein. The incubation medium in addition contained a final concentration of 40 m*M* K_2HPO_4, 40 m*M* KCl, 24 m*M* ADP and 20 m*M* succinate. Incubation was for 15 min at 30 °C and pH 7.4.



Peptide maps of a tryptic digest of normal human ceruloplasmin (left) and Wilson's ceruloplasmin (right). The conditions are given in the text. The arrows at positions A and B indicate the major peptide changes between the two.

ceruloplasmin is indicated too by the chymotryptic and tryptic peptide maps[7].

Bovine and normal ceruloplasmin do not interfere with oxygen consumption of mitochondria. Respiration was inhibited by ceruloplasmin from the Wilson in a concentration dependent way reaching a maximum of 85.8% with 80γ of the protein. These data are reviewed in the Table. The amine oxidase activity of Wilson's ceruloplasmin was enhanced somewhat by dialysis of serum against cupric-cuprous ions prior to isolation.

It would appear that the genetic defect which leads to the formation of ceruloplasmin of the Wilson type results in the exchange of 2 or 3 amino acid residues in the ceruloplasmin structure. Aside from minor differences, one notes the substitution of one neutral peptide in normal (position A) for a more basic peptide in the Wilson and the increased basicity of 3 peptides (position B) in the Wilson map. These changes, however, are reflected in the ability of the protein to bind copper, the electrophoretic mobility and the catecholamine and ascorbic acid oxidase activity in the homozygous state.

The genetically controlled structural alteration in ceruloplasmin decreases its oxidase activity and probably lays the groundwork for the deposition of copper which further damages the cells. BROMAN[8] has suggested that ceruloplasmin provides the copper for cytochrome oxidase by transfer of the entire prosthetic group containing the active (cupric) ions from the ceruloplasmin molecule, after some modification, to cytochrome oxidase. Thus the reduced level of cytochrome oxiduse seen in Wilson's disease can be explained[9]. Important is the observation in this work that Wilson's ceruloplasmin decreases mitochondrial respiration. In the mitochrondrion, the bonding between cupric ion and Wilson's ceruloplasmin is labilized. The released cupric ion is unavailable for a charge transfer reaction, does not participate in prosthetic group transfer but binds competitively, though nonfunctionally, with molecular oxygen or inhibits a number of vital enzymes through metal induced precipitations.

Zusammenfassung. Die primäre Struktur von Ceruloplasmin im homozygotischen Morbus Wilson unterscheidet sich vom Normalen durch eine grössere Zahl von basischen Aminosäuren. Das abnorme Ceruloplasmin hemmt die Mitochondrienrespiration; freie Kupferionen haben dieselbe Wirkung, was möglicherweise durch Auflösung des Kupfer-Eiweiss-Komplexes erklärt werden kann.

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Uptake of Labelled Histones by Isolated Perfused Rat Liver and by Various Organs of the Intact Mouse

One factor important in the regulation of transcription from DNA in the cell is thought to be nuclear histones[1]. This regulatory role is emphasized by recent observations which show that application of foreign histones interferes with the hormonal induction of enzymes in the intact animal[2] or in isolated perfused liver[3]. Evidently histones can act on transcription from DNA in a specific manner only if they are capable of entering the cell and the nucleus. This had been demonstrated so far only for *Vicia faba* roots[4]. In the present investigation, we have, therefore, followed the fate of labelled histones administered to intact mice or to isolated perfused rat liver.

Methods. Histones were labelled by perfusing an isolated regenerating rat liver with lysine ^{3}H. The histones were prepared from isolated chromatine as described elsewhere[3], and had a specific activity of 4.4×10^5 or 5.3×10^6 dpm/mg protein respectively for the 2 preparations. Labelled histones (1 or 0.3 mg) were injected i.v. into 2 adult mice (30 g weight) of the BALB/c strain. The animals were sacrificed 2 h later, various organs were removed, weighed and their activity was determined by liquid scintillation counting after dissolving an aliquot in hyamine. Nuclei were also isolated from liver and counted.

In another series of experiments, labelled histones diluted with different quantities of non-labelled rat liver histones were added to perfused rat liver preparations. The perfusion was carried out by a modification of Miller's technique[4]. The perfusate consisted of defibrinated rat blood diluted with $^1/_3$ of Ringer's solution. Non-labelled histones were added to the serum and the slight precipitate which formed with acid serum protein on standing was removed before perfusion when greater amounts of histones (15 and 20 mg) were utilized. Samples from liver or perfusate were removed 15, 60, 120 and (in most perfusions) 180 min after addition of the labelled histones. Radioactivity was determined and nuclei and histones were isolated from liver by conventional methods. TCA soluble activity was also measured in liver homogenate and in perfusate.

Results and discussion. 2 h after injection of labelled histones into mice, activity per g tissue is about the same for liver, lymphoid organs, kidney, intestine and serum (Table I). It is much lower in muscle, but since this organ represents a large part of the body, total activity in muscle is considerable. Liver nuclei contain small but significant amounts (a few μg) of labelled histones. Much of the radioactivity injected is still protein-bound after 2 h and about 75% of the activity present in liver can be precipitated with TCA.

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