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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Table I. Uptake of H^3 labelled liver histones by the intact mouse

	Activity/total organ (% of dose)		Activity/g organ (% of dose)	
	Experiment 1[a]	Experiment 2[b]	Experiment 1	Experiment 2
Blood serum[d]	7	5.5	7	5.5
Liver[c]	17	12	18	11
Liver nuclear fraction	1.4	0.7	–	–
(% activity of liver)	(6)	(6)		
Muscle[d]	≃27	37	1.9	2.5
Heart	–	0.5	–	5.7
Kidneys	4.0	2.3	20	10
Lung	0.8	1.0	10	9
Intestine	–	11	–	11
Spleen	1.2	1.4	18.5	12
Thymus	0.25	0.22	12.3	5.5

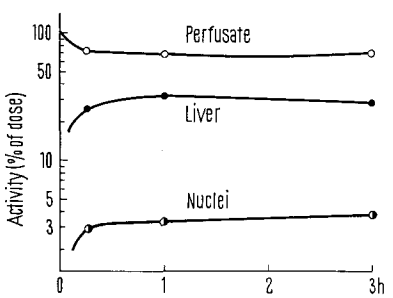
[a] Injection 0.21 μCi = 1 mg of histones, sacrifice after 2 h. [b] Injection 0.75 μCi = 0.3 mg of histones, sacrifice after 2 h. [c] About 75% of the activity still precipitable with 15% TCA. [d] Calculated by assuming a blood volume of 3% = 1 ml and a muscle weight of 50% of total body weight.

Table II. Uptake of H^3 labelled histones by isolated perfused rat liver

Amount of histones added (mg)	Perfusion time (h)	Hydrocortisone added after 1 h	Activity ratio			
			Total liver/ perfusate	Liver/ perfusate (g)	Nuclei/ liver	Nuclear histones/ liver
0.1	3	+	0.30	1.9	0.12	–
1	4	—	0.44	2.4	0.23	–
5	4	+	0.30	1.7	–	0.06
10	4	+	0.30	1.4	–	0.11
15	4	+	0.24	1.3	–	0.07

Uptake of histones by isolated perfused liver is rapid and essentially complete within 15 min after addition of the labelled material (Figure). Activity levels per g liver are somewhat higher than per ml perfusate and this difference becomes smaller as larger amounts of histones are given (Table II). Nuclei contain about as much activity as corresponds to their volume in the cell and most of this activity can be isolated as histones bound to chromatine. The amount of radioactivity in the chromatine does not vary greatly with the quantity of histones given and is not influenced by the application of hydrocortisone. It can be estimated that about 50 μg of foreign histones are bound per 1 mg of endogenous DNA when perfusion was carried out with 15 mg of histones. 25–30% radioactivity in liver and 30–50% in perfusate is not precipitable by TCA and this percentage is independent of the length of perfusion or the amount of histones given.

As in all experiments with labelled macromolecules in vivo it must be asked whether the histones have been taken up as such or only after degradation to the constituent amino acids. It is postulated that histones have been taken up as such for the following reasons. Re-utilization of lysine is small. Only 2.5% of the dose is found in albumine after a 3 h perfusion, but labelled histones are found in the nucleus already after 15 min. Moreover, synthesis of histones in normal liver is extremely small (less than 0.01% of ^3H lysine). The material not precipitable with TCA probably still represents histones or large polypeptides since its amount remains constant during perfusion. It is therefore concluded that histones can enter the mammalian cell nucleus[6].



Uptake of labelled histones (0.1 mg) by isolated rat liver perfused with 35 ml of defibrinated blood.

Résumé. Des histones marquées au H^3 ont été administrées à des souris par voie intrapéritonéale. La pénétration d'histones dans le foie isolé en perfusion est rapide. Les noyaux contiennent une quantité plus ou moins proportionnelle à leur volume dans la cellule et la majorité de l'activité a pu être isolée comme histones liées à la chromatine.

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