Measurements of the Ca^{2+} concentration in these fibers show not only significant differences between fibers from the 3 specimens studied but also between fibers from the same muscle bundle. Another clear-cut feature of these results is that the Na and Ca content of fibers from specimen A parallel each other. Both the Na and Ca values are high, and this is in striking contrast to the low Na and Ca values found in fibers from specimens B and C. The Mg^{2+} concentration seems to be quite steady and not very different from the findings of PAGE, MOBLEY and LEWIS[7].

The salient feature of the present results is that differences in ion concentration between fibers from the same muscle bundle show themselves when fibers are freshly dissected in artificial sea water containing HCO_3^- as buffer. So far as the mean values of the ionic content of these fibers are concerned, they are in fairly good agreement with those reported by other workers, as shown in Table II. MCLAUGHLIN and HINKE[2] found barnacle fibers (in 1964) to have a high Na content. The reason for this divergence could have been the use by them of *25 mM Tris* NO_3 as buffer, as well as allowing fibers to soak for varying periods in artificial sea water containing such a high concentration of *Tris*. This is thought to be so because there is evidence from experiments with barnacle fibers that the presence of *Tris* in the bathing medium leads to prolongation of the equilibration time of injected ^{22}Na (BITTAR et al. unpublished).

GAYTON et al.[6] have suggested that a low Na and K content in fibers is an indication of aging. However, the distribution of Na and K in relation to aging is supposedly reciprocal, i.e. there is a gain in Na and a loss in K. It would appear (to me) more likely that a low Na and K concentration is a characteristic of fibers from moulting barnacles. This possibility is now being investigated[9].

Table II. Various estimates of ionic concentrations in Balanus muscle fibres (m*M*/kg fiber water)

	Na	K	Ca	Mg	Cl
1. HAGIWARA, CHICHIBU and NAKA[3]	21	157			35
2. MCLAUGHLIN and HINKE[2a]	81 (in 1964)	168			10
	51 (in 1965)	143			
3. BRINLEY[4]	21	150			
4. BEAUGÉ and SJODIN[5]	21	167			
5. GAYTON and HINKE[1]					75.1 and 66.8
6. GAYTON, ALLEN and HINKE[6b]	21.8	160			36
7. ASHLEY[8]			0.78 m*M*/kg wet wt.		
8. PAGE, MOBLEY and LEWIS[7]				50 m*M*/kg dry wt.	
9. This paper	23.3	133	2.0	10.8	28.2

[a] Uncorrected for extrafiber space (6% of fiber water). [b] Corrected for extrafiber space (6% of fiber water).

Zusammenfassung. Es werden Analysenresultate über den Ionengehalt von einzelnen Muskelfasern der Entenmuschel, *Balanus nubilus*, mitgeteilt. Es wurden Unterschiede nachgewiesen in der Ionenkonzentration verschiedener Fasern, die von demselben Muskelbündel isoliert worden waren.

E. E. BITTAR

University of Wisconsin, Department of Physiology, Service Memorial Institutes, Madison (Wisconsin 53706, USA), 11 January 1971.

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Penicillamine Induced Changes in Growing Rats. II. Liver Parenchymal Cell

D-Penicillamine is a chelating agent almost specific for copper, binding the metal present in the body[1] and inhibiting the intestinal uptake[2]. Therefore, penicillamine is generally applied for prophylaxis and therapy of Wilson's disease[3,4]. This autosomally inheritable copper storage disease is characterized by a deficiency of ceruloplasmin and deposits of copper in liver, brain, kidney and cornea[5]. The human body normally contains about 100 mg of copper, 98% of which is bound to ceruloplasmin, an α_2-globulin. This copper-protein complex is not toxic and acts as a ferrooxidase in synthesizing iron-containing enzymes in the respiratory chain[6,7]. Copper is a constituent of oxidases, e.g. xanthine oxidase, uricase, amine oxidase of microbodies[8] and cytochrome oxidase of mitochondrial cristae[9]. All these enzymes, except amine oxidase, function as so-called terminal oxidases in the last step of biological oxidation processes. Cytochrome oxidase transfers the hydrogen from substrate to oxygen at the end of the respiratory chain. Uricase and xanthine oxidase catalyse the terminal oxidation of purines, and are able to interact themselves with molecular oxygen[9].

The present paper deals with quantitative ultrastructural changes in rat liver cells after long-term application of Penicillamine, examined by means of stereological methods. Thereby, mitochondria and microbodies, the main sites of copper-containing enzymes, were especially considered.

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Material and methods. Six-week-old male Wistar rats were given 200 mg D-Penicillamine (Distamine, DISTA, Liverpool) in tap water daily for 7 weeks intragastrically. Liver tissue of 3 treated and 3 control animals was fixed in 1% s-collidin buffered OsO_4 at 4 °C and embedded in Epon 812 after dehydration in graded alcohols. 12 tissue blocks of each animal were prepared. Morphometric procedure basically followed the method described by WEIBEL[10]. 3 blocks per animal were taken for random-sampling and ultrathin sections (interference color: silver). Three electron micrographs per section were made at a magnification of ×5000 and analysed with a quadratic lattice of 841 test points[11]. In addition, 3 micrographs at a primary magnification of ×10,000 were enlarged with the multipurpose test screen[11]. The following parameters were considered:

Compartment, organelle	Symbol
Nucleus	N
Extracellular space	
Amorphous cytoplasmic components	
Rough endoplasmic reticulum	RER
Smooth endoplasmic reticulum	SER
Glycogen	GLY
Fat	FAT
Mitochondria	M
– Mitochondrial cristae	MC
– Mitochondrial outer membrane	MO
Microbodies	MB
Golgi field	GF
Lysosomes	LY

Abbreviations: N_A, number of particles within the unit area (cm^{-2}); N_V, numerical density, indicating the number of particles in the unit tissue volume (cm^{-3}); V_V, volume fraction of tissue occupied by a compartment (cm^3/cm^3); I_L, density of intersections on test line length (cm^{-1}); S_V, surface density, measuring the surface of membranes in the unit tissue volume.

Statistical evaluation included mean values, standard deviation and standard error (S.E.). The significance was calculated by means of Student *t*-test. All these calculations were done with the aid of a Olivetti Programma 101 computer.

Results. Body weight of control rats increased about 125% during the time span of the experiment, whereas Penicillamine treated rats gained only about 25% of weight.

Ultrastructure of hepatocytes after long-term Penicillamine treatment did not differ markedly from normal liver parenchymal cells as described previously[12,13]. Especially, no objective changes were noted in SER, RER, lysosomes, bile canaliculi, or microvilli. Microbodies and mitochondria seemed to be enlarged (Figures 1 and 2), the latter showing very numerous cristae and an unaltered electron density of their matrix.

Morphometric analysis revealed significant changes of mitochondria and microbodies only (Figures 1 and 2). While the single mitochondrial volume (V_{VM}/N_{VM}) only slightly increased (13%, Figure 3), the values for surface density of outer membranes (68% augmentation) and cristae were considerably higher (84% augmentation, Figure 4). This increase in mitochondrial surface density per unit volume liver tissue (S_{VMO}, S_{VMC}) was the same in relation either to a single mitochondrion (S_{VMC}/N_{VM}) or to a single hepatocyte (S_{VMC}/N_{VNH}, S_{VMO}/N_{VNH}, Table II). Volume density of microbodies per hepatocyte V_{VMB}/V_{VH}) showed nearly twice the initial values (41% augmentation, Figure 5). All the morphometric results obtained are summarized in Table I and II.

Discussion. The most striking morphometric findings after long-term Penicillamine application are a doubling of volume density of microbodies per hepatocyte and a massive augmentation of the surface of mitochondrial cristae and outer membrane (Figures 1 and 2). Microbodies are known to contain 2 copper enzymes involved in intermediary metabolism, i.e. uricase and xanthine oxidase[8]. Therefore, a relation between serum copper level and volume of microbodies is probable. In a morphometric study, the desert rat, having available only the product of cellular oxidative processes as sole source of water, showed a large number of small microbodies. In comparison, Wistar rat liver cells revealed less numerous but larger microbodies[14]. Furthermore, an increased

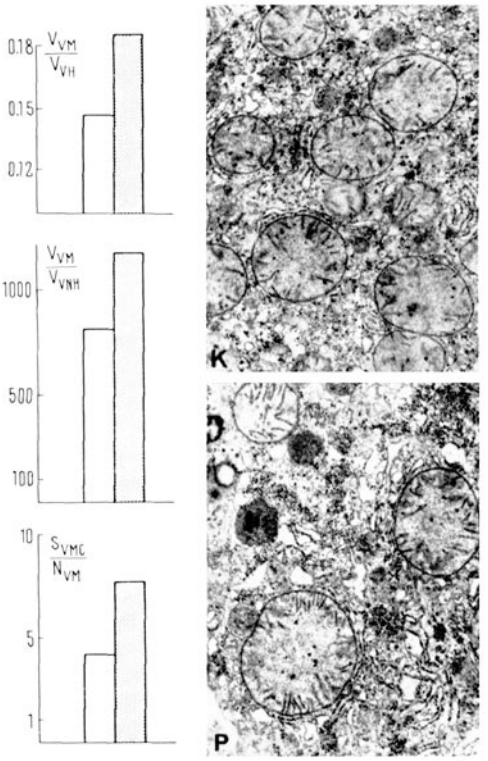

Fig. 1. V_{VM}/V_{VH}, volume density of mitochondria per hepatocyte slightly increased after Penicillamine treatment (black column). White column = control. V_{VM}/N_{VNH}, absolute volume of mitochondria per hepatocyte somewhat higher after Penicillamine treatment (black column). White column = control. S_{VMC}/N_{VM}, surface density of mitochondrial cristae per mitochondrion nearly doubled after Penicillamine treatment (black column). White column = control. Electron micrographs comparing liver mitochondria of control rat (K) and Penicillamine-treated rat (P). Note greater number of cristae in enlarged mitochondria after Penicillamine treatment (P). ×13,333.

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amount of small microbodies was reported after application of acetylsalicylic acid[15]. This substance is said to be metabolized by enzymes of microbodies[16]. Such alterations of microbodies suggest that high functional demands may influence shape and volume of microbodies.

Two interpretations for our findings of altered microbodies after Penicillamine treatment are given: The formation of microbodies is either directly inhibited by the copper deficiency, or functional efficiency of enzymes in this organelle becomes poor. As a consequence of this latter possibility, the turnover of microbodies would be slower and the life span of the population prolonged. The presence of nucleid favors the second suggestion. Since nucleid contains uricase and xanthine oxidase[8], one would expect a lack of nucleid after long-standing Penicillamine treatment.

In our experiment, mitochondria, moreover, revealed a conspicuous increase in the surface of their cristae. A functional relationship between this alteration and the cuproenzyme cytochrome oxidase, localised in mitochondrial cristae[17] can be assumed. Treatment with Cuprizone, an other chelating agent for copper, results in an increase in cristae of normal shaped mitochondria and leads to the formation of giant mitochondria[18]. The enlargement of the mitochondrial outer membrane is supposed to be functionally related to amine oxidase[18]. This copper-containing enzyme of the outer membrane[19] may be altered by the Cuprizone induced copper deficiency[18].

On the other hand, a loss of mitochondrial membranes in giant mitochondria after Cuprizone treatment is reported[18]. In our experiments with Penicillamine, however, as well as after copper-deficient diet[17], the enlargement of mitochondria was not conspicuous and a loss of membranes could not be confirmed. These differences suppose that the 2 copper chelating agents, Penicillamine and Cuprizone, must have different sites of action on the subcellular level.

Giant mitochondria have been described in many other deficiency conditions, such as riboflavin deficiency[20], essential fatty acid deficiency[21]. After Penicil-

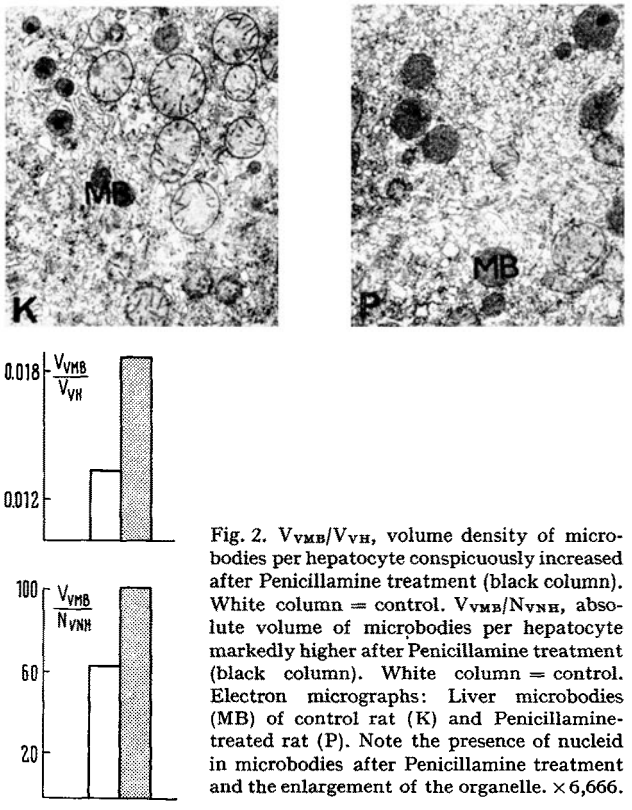

Fig. 2. V_{VMB}/V_{VH}, volume density of microbodies per hepatocyte conspicuously increased after Penicillamine treatment (black column). White column = control. V_{VMB}/N_{VNH}, absolute volume of microbodies per hepatocyte markedly higher after Penicillamine treatment (black column). White column = control. Electron micrographs: Liver microbodies (MB) of control rat (K) and Penicillamine-treated rat (P). Note the presence of nucleid in microbodies after Penicillamine treatment and the enlargement of the organelle. ×6,666.

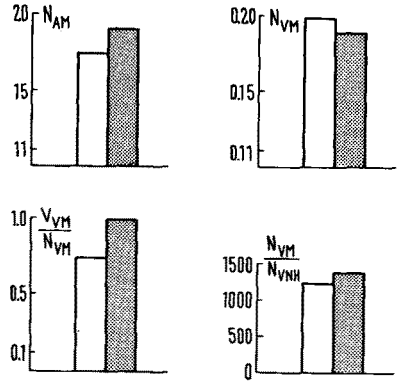

Fig. 3. N_{AM}, number of mitochondria within unit area. N_{VM}, number of mitochondria per unit liver tissue. No significant change after Penicillamine treatment (black column). White column = control. V_{VM}/N_{VM}, single mitochondrial volume slightly increased after Penicillamine treatment (black column). N_{VM}/N_{VNH}, number of mitochondria per hepatocyte not significantly influenced by Penicillamine treatment (black column, white column = control).

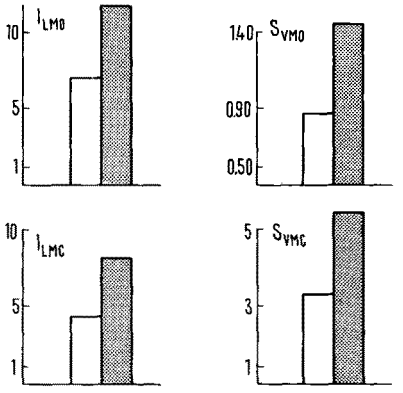

Fig. 4. I_{LMO}, density of intersections on test line length with mitochondrial outer membrane. S_{VMO}, surface density of mitochondrial outer membrane per unit volume liver tissue: Significant augmentation after Penicillamine treatment (black column). White column = control. I_{LMC}, density of intersections on test line length with mitochondrial cristae. S_{VMC}, surface density of mitochondrial cristae per unit volume liver tissue: Significant augmentation after Penicillamine treatment (black column).

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Table I. Morphometric basic values

Symbols	Control rats	S.E.	Penicillamine treated rats	S.E.	Probability
N_{ANH}	4.366	0.344	3.880	0.087	not significant
V_{VNH}	0.025	0.002	0.013	0.007	not significant
N_{AM}	17.656	1.395	19.370	0.537	not significant
I_{LMO}	7.245	0.802	11.987	0.304	<0.01
I_{LMC}	4.599	0.674	8.477	0.325	<0.005
N_{AMB}	3.645	0.198	4.777	0.501	not significant
V_{VMB}	0.013	0.007	0.019	0.001	<0.025
V_{VRER}	0.201	0.015	0.179	0.029	not significant
V_{VSER}	0.140	0.018	0.100	0.018	not significant
V_{VGF}	0.004	0.001	0.005	0.002	not significant
V_{VH}	0.862	0.012	0.839	0.013	not significant

Table II. Correlated values

Symbols	Control rats	Penicillamine treated rats
N_{VNH} ($\times 10^6$)	0.000180	0.000154
V_{VH}/N_{VNH} (μ^3)	4791.255	5451.261
V_{VNH}/N_{VNH} (cm^3)	291.883	328.792
V_{VNH}/V_{VH} (cm^3/cm^3)	0.060920	0.060307
V_{VM}/V_{VH} (cm^3/cm^3)	0.149665	0.186710
V_{VM}/N_{VNH} (cm^3)	0.519412	1.069232
N_{VM} ($\times 10^{12}$)	0.198452	0.192141
V_{VM}/N_{VM} (μ^3)	0.754162	0.971734
N_{VM}/N_{VNH}	1102.511	1247.668
S_{VMC} (m^2/cm^3)	0.845760	1.488171*
S_{VMC}/N_{VNH} (μ^2)	4698.666	9663.448*
S_{VMO} (m^2/cm^3)	0.372643	0.616182*
S_{VMO}/N_{VNH} (μ^2)	2070.238	4001.181*
S_{VMO}/N_{VM} (μ^2)	1.877758	3.206942*
N_{VMB}	0.066937	0.080569
V_{VMB}/N_{VMB} (μ^3)	0.173013	0.197768*
N_{VMB}/N_{VNH}	371.872	523.175
V_{VMB}/V_{VH} (cm^3/cm^3)	0.013428	0.018978*
V_{VMB}/N_{VNH} (μ^3)	64.338	103.467*
V_{VLY}/V_{VH} (cm^3/cm^3)	0.003701	0.005397
V_{VRER}/V_{VH} (cm^3/cm^3)	0.205552	0.186108
V_{VRER}/N_{VNH} (μ^3)	984.855	1014.655
V_{VRIBO}/N_{VNH} (μ^3)	286.661	427.766
V_{VSER}/V_{VH} (cm^3/cm^3)	0.143172	0.103618
V_{VSER}/N_{VNH} (μ^3)	685.977	564.922
V_{VGLY}/V_{VH} (cm^3/cm^3)	0.176923	0.129044
V_{VGLY}/N_{VNH} (μ^3)	847.683	703.545
V_{VGF}/V_{VH} (cm^3/cm^3)	0.004609	0.005827
V_{VGF}/N_{VNH} (μ^3)	22.083	31.772
V_{VFAT}/V_{VH} (cm^3/cm^3)	0.003838	0.003296
V_{VFAT}/N_{VNH} (μ^3)	18.388	17.974

* Correlated values resulting from statistically significant basic values.

lamine application, however, this mitochondrial transformation cannot be detected. The mechanisms responsible for the increase of mitochondrial cristae are not yet understood. WILSON and LEDUC[21] interpreted the mitochondrial enlargement in essential fatty acid deficiency as a negative feed-back mechanism: The altered molecular architecture of mitochondrial membranes is presumed to be due to a replacement of the lacking essential fatty acids by unsaturated ones. As a result, the oxidative phosphorylation, and consequently the production of ATP, would be diminished. Thus, the main source of cell energy would be exhausted. This lack of energy would then act as a trigger for the growth of mitochondrial substance forming additional cristae.

Analogously to this hypothesis, Penicillamine-induced increase in mitochondrial cristae could be interpreted as a defective compensation of inefficient membrane-bound enzymes.

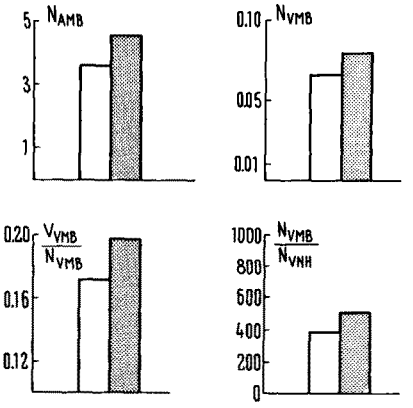

Fig. 5. N_{AMB}, number of microbodies within unit area. N_{VMB}, number of microbodies per unit volume liver tissue: No significant change after Penicillamine treatment (black column). White column = control. V_{VMB}/N_{VMB}, single volume of microbodies significantly increased after Penicillamine treatment (black column). White column = control. N_{VMB}/N_{VNH}, number of microbodies per hepatocyte: No significant change after Penicillamine treatment (black column).

Zusammenfassung. D-Penicillamin als Kupferchelator bewirkt im Langzeitversuch eine isolierte Veränderung der stereologischen Parameter der Mitochondrien und der «microbodies». Zusammenhänge mit der Aktivitätshemmung kupferhaltiger Oxydasen in den beiden Zellorganellen werden vermutet.

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An Electrophysiological Study of Human Foetal Cardiac Muscle

Although extensive information is available on the transmembrane action potential of heart muscle from many species, the findings are of limited value in assessing the response to disease or drugs in man. The study of human transmembrane action potentials has been restricted to records obtained with flexible microelectrodes in situ during cardiac surgery[1,2] and in vitro using pieces of atrium or ventricle excized during cardiac surgery[3-7]. These studies have been somewhat limited, since the heart is often hypothermic at surgery for operative reasons and biopsy specimens are usually obtained from hearts damaged by rheumatic fever or other diseases.