ZINC CONTENT IN THE SERUM AND CARBONIC ANHYDRASE INHIBITION

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It is well known that the activity of certain cellular enzymes is related to the presence of a zinc ion in their active center, and that metal bound to it accounts for inactivation of the enzyme (3, 7, 23, 24). The carbonic anhydrase (CAH), the lactic acid-, alcoholic- and glutamate-dehydrogenase, carboxypeptidase etc. fall within the group of zinc-metal enzymes alluded to. As early as 1940 Keilin and Mann (10) demonstrated that CAH of the erytrocytes contains 0.33% zinc, and moreover that the sulfonamides account for inhibition of its activity (10, 22, 23). A reversible inactivation of CAH is obtained under the effect of BAL, cyanides, sulfides, sulfonamides etc., all of them zinc bound (24). A rapid and intense exchange of zinc ions is observed between plasma and erythrocytes under physiological and pathological conditions alike (18). In 1950 Roblin and Clapp derived the acetazolamide which exerts a strong inhibitory effect upon the activity of CAH in the kidney and thus produces diuresis. The mechanism of acetazolamide inhibition of carbonic anhydrase is not thoroughly clarified. It is quite possible that it occurs through the zinc ion linkage to the active CAH center, but the other routes by which inactivation of the metal enzymes is generally accomplished are not excluded (4). Nevertheless, it is beyond doubt that the activity of CAH is conditioned by its zinc link (13), and furthermore, that the diuretic effect of acetazolamide is secondary to CAH inhibition in the kidneys (2, 19, 22).

The goal of the present work is to prove exactly what is the influence of acetazolamide exerted upon the zinc in the serum and upon the zincuria in individuals treated with it. It is known that the hydrochlorthiacide (neodehydratin — ND) and chlorthalidone (saluretin — S) exert an insignificant effect on CAH in vitro compared with acetazolamide (dehydratin — D).

Material and Methods

A series of 23 patients underwent investigation. In 18 of them (group A) the zincemia was determined prior to receiving D and daily, over a period of 3 days, during which period the patients received 2×250 mg dehydratin per 24 hours. In 9 patients of this group the zincuria in the 24-hour urine was also determined. Four additional patients were investigated in the same fashion, undergoing immediately prior to the experiment a treatment with diuretics — with dehydratin — 2 cases, with neo-dehydratin — 1 and with

saluretin — 1 (group B). One patient was also studied in whom neo-dehydratin was applied in doses of 50 mg per 24 hours.

The patients received in addition 2—3 gr potassium chloride, and those with cardiac insufficiency digitalis as well. Drugs influencing the metabolism of zinc were not administered.

The zinc was determined after the method of Wolf, with ditison, as modified by Rechenberger (17, 26, 27).

Results

In all the 18 patients of group A a decrease was established of the serum zincemia and increase of the zincuria in compairson to initial values. Zincemia was acutely lowered as early as receiving the first 500 mg D; in the following two days the decrease of zincemia was moderate. Occasionally, a sbustantial decrease of zincemia was also observed (Table 1). On the whole, the zincemia was decreased for the total number of cases in the average from 144 ± 23 $\gamma\%$ to 96 ± 19 $\gamma\%$, i. e. reaching evident hypozincemic concentrations. In the 9 patients group zincuria was increased in the average from 384 ± 93 $\gamma\%$ to 1029 ± 481 $\gamma\%$ for 24 hours.

The results are statistically reliable (Table 1).

In the four patients of group B, the zincemia was maintained unchanged, whereas the zincuria and diuresis were insignificantly raised and were statistically unreliable (Table 2). The zincemia in the patient treated with neo-dehydratin was unaltered. A strict parallelism between the decrease of zincemia, raise of zincuria and diuresis in the patients receiving dehydratin was not established. However, alterations in the zincemia and zincuria were recorded at the time when obvious increase of diuresis was produced by dehydratin, i. e. when its inhibitory effect upon the renal CAH was manifested (Tables 1 and 2).

Discussion

The zinc in the serum is found in two forms: firmly bound zinc = 34% of the total zinc, and unstably bound zinc, representing a metal protein complex — 66% (24). Under pathological conditions, the changes in zincemia occur at the expense of changes in the unstable fraction (8, 24). It could be assumed by analogy that the dehydratin, provided with a sulfonamide radical, binds part of the labile zinc, extracts it and emits it with urine. The increase of zincuria is a further support of a similar hypothesis.

Presumably, with blocking of renal CAH, occurring simultaneously with the above process, the kidney increases its permeability for the zinc ion in a fashion that zincemia appears to be the sequela of hyperzincuria induced by contemporaneous increasing of diuresis. Normally, scarcely 2—3% of the alimentary zinc is separated in the urine (25), and the zincuria appears not to be dependent upon the 24-hour diuresis (9).

It is established that a major part of alimentary zinc is excreted via the intestines (6, 21, 20, 24).

Having established approximately a three-fold increase of zincuria under the effect of dehydratin, a quantity hardly exceeding 10% of the alimentary zinc (bearing in mind that normally about 30% of the zinc received with

Table I
(group A)

Zincemia and Zincuria Prior to and After Receiving Dehydratin (P. R. B.)

Name history of Illness No.	Diagnosis	Zincemia before	γ % after	Zincuria before	γ/24 h after	Diuresis before	ml/24 h. after
S. K. S. 12699 14. 10. 64	Carcinoma bronchi dex. Ca hepatis metastatica	140	116	—	—	—	—
N. T. E. 13020 22. 10. 64	Asthma bronchiale Emphysema pulmonum	130 132—131	78	—	—	—	—
T. V. I. 13588 4. II. 64	Rheumatism. Vitium cordis (Stenosis et insuf. valv. mitr.)	90	80.5	—	—	—	—
I. A. M. 15107 9. 12. 64	Bronchitis chronica. Emphysema pulm. Cor pulmonale. Decomp. II	106 116—111	103	—	—	—	—
M. A. S. 15693 22. 12. 64	Bronchitis chronica Emphysema pulm. Cor pulmonale Decomp. III	183 193—188	77 83—80	—	—	—	—
G. D. G. 393 9. I. 65	Bronchitis chron. Emphysema pulmonum.Cor pulmonale. Decomp. II	155	109	—	—	—	—
D. I. 1644 5. 2. 65	Bronchitis chron. Emphysema pulm. Cor pulmonale	119 126—122.5	100 64—82	—	—	—	—
Z. P. Z 2091 15. 2. 65	Myocardiosclerosis. Arrythmia absoluta. Emphysema pulm. Decomp.II	193 161—177	106 100—103	—	—	—	—
A. H. K. 2277 19 2. 65	Atherosclerosis aortae Myocardiosclerosis. Hypertonia. Decomp. III	128	48 60—54	—	—	—	—
S. P. R. 2712 23. 2. 65	Bronchitis chron. Emphysema pulm. Cor pulmonale. Decomp. I	135 141—138	80.5	351 464—407.5	272 592—428.3 421	900 900—900	1640 920—1250 1190
R. A. I. 4170 10. 3. 65	Bronchitis chron. Emphysema pulm. Cor. pulmonale. Decom. I.	129 145—137	90 97—102 119	— 309—337 365	1083 1420—1011.6 532	600 320—446 420	1290 1340—1076 600

Name history of illness No.	Diagnosis	Zincemia before	γ % after	Zincuria before	
K. I. R. 4207 31. 3. 65	Bronchitis chron. Cor pulmonale. Emphysema pulm. Decomp. III	168 144—156	119 106—111.3 109	356 703—529.5	2
D. A. A. 4626 8. 4. 65	Bronchitis chron. Cor pulm. Emphysema pulm. Myocardiosclerosis. Decomp. II	180 151—165.5	97 97—165.5 64	288 213—250.5	
D. P. S. 4705 10. 4. 65	Myocardiosclerosis atherosclerotica. Arrythmia absoluta. Bronchitis chr. Emphysema pulm. Decomp. II	160 180—170	97 129—188.3 129	504 469—486.5	
I. Y. S. 5420 26. 4. 65	Vitium cordis. Insuf. et stenosis valvulae mitr. aortae. Decomp. III	145 129—145 161	106 145—125.5	419 256—337.5	1
E. N. Ch. 5468 24. 4. 65	Myocardiosclerosis atherosclerotica. Decomp. III	161 136—148.5	122 116—119	421	2 1
A. I. I. 6795 28. 5. 65	Rheumatismus acutis Pheumocarditis. Vitium. Decomp. III	161 177—169	154 84—114.6 106	186 309—247.5	2
G. K. K. 6779 28. 5. 65	Bronchitis chronica Emphysema pulmonale. Cor pulm. Decomp. II	126 129—127.5	129 64—80.3 48	409 480—444.5	1 2
Statistical elaboration of results		X ± S144 ± ± 23γ	96 ± 19γ	384 ± 93γ	
Average error (of standard deviation)		Sx 5.3	4.4	31	
P according to "t" criterium of Student			P=0.1%	P=<	
d > D		48>47.9		645>(	

Zincemia and Zincuria Prior to and After receiving Dehydratin Foll
previous Treatment with Diuretics

Name History illness	Diagnosis	Zincemia γ %		Zincuria γ/2	
		before	after	before	
H.N.R 5849 5. 5. 65	Vitium cordis. (insuf. sten. valv. mitr.) Decomp. III	161 154—157.5	145 129—138.3 141	432 523—477.5	38 77 10
N.N.Z. 5947 7. 5. 65	Morbus hypertonicus Cor hypertonicus. Decomp. II	154 167—161	145 129—141.6 151	117 378—247.5	30 24 25
T.V.I. 6789	Rheumatismus. Vitium (stenosis	97	128	301—227	39

food is resorbed), it is obvious that zincuria alone could not explain the prompt and substantial decrease of seral zinc.

The following mechanisms might be considered:

a) the dehydratin blocks zinc resorption in the intestines;

b) the dehydratin stimulates zinc excretion into the intestines and other organs, and in their secretions respectively.

Neither of the presumptions stated, however, could explain the rapid and substantial decrease of zinc in the serum and accordingly increase of zincuria.

c) It may be assumed that under the effect of dehydratin, directly or indirectly, through CAH inhibition in the erythrocytes of other cells, a redistribution of zinc takes place from the plasma towards some cellular elements. Under the aspect just outlined, it is of interest to trace out the changes in CAH and in the zinc content of erythrocytes prior to and after application of dehydratin. Verification of the relative importance of these assumptions is forthcoming.

The reduction of zincemia is not a sequela of digitalis and potassium chloride treatment, as a fall of zincemia level is also recorded in individuals not subjected to a similar treatment, and by virtue of the fact that digitalis treatment precedes the experiment with D etc.

The circumstance that zincuria rises as a result of the effect of a potent inhibitor exerted upon exclusively cellular enzyme, such as the CAH, for whose activity the zinc ion is essentially indispensable, proves that zincuresis does not constitute an ordinary filtration process, but rather a process related to the function of cellular structures.

Our observation that zincemia and zincuria are not significantly changed, provided previous to the application of dehydratin the patients underwent treatment with some of the diuretics, is a further confirmation of this idea. Presumably, in these irstances the cells of the uriniferous tubules resist the increase of zincuria. Whether the causes for the reduction of diuresis during prolonged application of diuretics also exerts certain effect on the zinc secretion from the kidneys is a question yet unanswered. Furthemore, the seral zinc in the four patients, treated with diuretics previous to the application of dehydratin, was retained unaltered. Two of them, previous to the acute experiment with dehydratin received also D. Very likely, during a prolonged treatment with dehydratin the organism puts on regulatory mechanisms which a) gradually restore normal zincemia and b) render the latter resistant to a new dehydratin block. Such a tendency is also marked in some patients as early as after the first day of application of dehydratin, being its initial dose the most efficient (Table 1, history of illness № 4170, history of illness № 4705 etc.).

The negative result of the experiment with dehydratin in the two patients, previously treated with neo-dehydratin and saluretin, as well as in the single patient investigated with ND alone is quite indicative.

Most probably, the diuretics listed are not endowed with hypozincemizing effect. The latter fact is in accordance with their well known property, namely, that they do not inhibit the activity of CAH or they bring about merely an insignificant inhibition as compared to D, in vitro.

On the other hand, however, Pulver and assoc. suppose that the chlorthiacide and chlorthalidone in vivo are split in powerful CAH inhibitors (16).

If the latter holds true, it is likely that under the effect of their metabolites in the patients of our series treated previously with ND and S, changes might occur analogous to those achieved during prolonged treatment with dehydratin, substantiating the resistance to the acute D blow. Thus, an explanation of the changes observed in the serum zinc and zincuria might be found. We are not able to answer the question whether in this case a competetive effect is concerned on behalf of the diuretics discussed, or on behalf of their metabolites upon the dehydratin.

The interpretation of our results concerning the effect of dehydratin upon zincemia is in the sense that it brings about a reduction of serum zinc mainly through inhibiting the CAH in the kidneys with simultaneous diuresis and zincuria increase.

Our experiments did not furnish an answer to the question up to what extent does the CAH of erythrocytes and other tissues participate in the sense of zinc re-distribution between plasma and cells.

Inferences

1. Dehydratin applied in a dose 2×250 mg/24 hours for a duration of 3 days, produces statistically reliable reduction of the previously normal zinc level in the serum.

2. A simultaneous raise of zincuria is also observed.

3. Individuals with a past history of diuretic treatment (neo-dehydratin, saluretin, dehydratin) do not reveal changes in the serum zinc and in zincuria subsequent to charging with dehydratin in the doses indicated. It is probable that the organism is rendered resistant to a new dehydratin „blow", after having overcomed by way of a proper regulatory route the originally produced by D hypozincemia.

Our observations on the occurrence of resistance after application of other diuretics (ND and S) are very limited and hence, a definitive conclusion concerning the mechanism of their action is not warranted.

4. It is supposed that the effect of dehydratin on the metabolism of zinc is achieved through influencing the CAH activity, as changes in the serum zinc and zincuria are noted merely provided an increase of diuresis occurs under the effect of dehydratin which blocks the carbonic anhydrase.

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СОДЕРЖАНИЕ ЦИНКА В СЫВОРОТКЕ КРОВИ И ИНГИБИЦИЯ КАРБОАНГИДРАЗЫ

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РЕЗЮМЕ

Авторы находят, что после дачи дегидратина (ацетацоламид) — 2× 250 мг/24 ч., в течение 3 дней у 18 больных с показаниями для принятия этого медикамента, наступает статистически достоверное снижение количества цинка в сыворотке крови и у 9 исследованных больных — увеличение цинкурии. После обсуждения возможных механизмов этих изменений, авторы толкуют полученные результаты в смысле, что дегидратин оказывает гипоцинкемизирующий эффект путем подавления карбоангидразы в почках, но не исключают возможности соучастия карбоангидразы других тканей, соотв. таковой эритроцитов.