IRON METABOLISM DURING EXPERIMENTAL ADMINISTRATION OF COMPLEXONS

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UDC 615.9-085.732.8-07:616-008.92-074

The complexons which are administered for therapeutic purposes form stable complexes not only with toxic metals, but also with certain metals essential to the living organism. Stable complexes are formed with iron, especially with trivalent iron [3, 7].

Several authors have demonstrated an increase in the excretion of iron during complexon therapy [2, 4, 5, 9, etc]. It has been reported [8] that if $CaNa_2$ ethylenediaminetetraacetic acid ($CaNa_2$ EDTA) is added to the diet for a month, the experimental animals are very retarded in weight and they develop anemia with a considerable fall in the hemoglobin concentration. However, during the treatment of lead poisoning with $CaNa_2$ EDTA in man, large amounts of calcium and copper were found in the urine [7], whereas the iron content was unchanged.

Data in the literature demonstrate changes in the iron metabolism in the course of complexon therapy, but these results relate mainly to one complexon – $CaNa_2$ EDTA, and only in one or two papers [2, 3] can references be found to the effect of $CaNa_2$ diaminocyclohexanetetraacetic acid and $CaNa_3$ diethylenetriaminopentaacetic acid ($CaNa_3$ DTPA) on the excretion of iron.

The object of the present investigation was to compare the effects of a series of complexons on the excretion and distribution of iron in experiments on animals.

EXPERIMENTAL METHOD

Experiments were carried out on 117 female rats weighing 190 ± 15 g.

As indicator of the iron, it was labeled with Fe^{59} in the form of iron ascorbinate, for several authors [1] have shown that this compound of iron is absorbed by the body better and more rapidly. The solution of iron ascorbinate was injected intraperitoneally into the animals in a dose of 4 μCi.

The effect of the following complexons on the distribution and excretion of iron was studied: $CaNa_2$ EDTA and $CaNa_3$ DTPA, the $CaNa_2$ salts of the 2,2'-diaminodiethyl ester of tetraacetic acid ($CaNa_2$ DEETA), the 2,2'-diaminodiethyl sulfide of tetraacetic acid ($CaNa_2$ DESTA), and ethylenediamine-bis-Na_2-isopropylphosphinic acid (phosphicine), and also 2,3-dimercaptopropanesulfonate Na (unithiol). The complexons were injected intraperitoneally in doses of 30 mg per rat daily for 3 days.

Two series of experiments were performed. In series I the animals received complexons 24 h after administration of iron. The object of performing the investigation in this way was to ascertain the effect of the complexons on the face of the iron which was not stored in the depots. In series II the effect of the complexons was investigated 11 days after administration, i.e., after the iron had taken part in the metabolic reactions and had become completely absorbed by the organism [1].

The radioactivity of 24-hour samples of stools and urine, and also the radioactivity of the organs was measured as γ-radiation by the method of comparison with a standard, prepared by addition of the original solution of iron ascorbinate to the test sample.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1. In the experimental conditions described, iron is normally excreted mainly by the intestine. Administration of all the test preparations led to a considerable

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TABLE 1. Excretion of Fe^{59} (in % of Amount Administered and of Control)

Complexon administered	No. of animals	Substrate	1st day	2nd day	3rd day	During 3 days: as % of amount administered	During 3 days: as % of control	During 3 days: total in urine and stools: as % of amount admin.	During 3 days: total in urine and stools: as % of control
Control	9	urine	0,02	0,08	0,047	0,147	100	1,95	100
		stools	0,82	0,51	0,47	1,80	100		
Unithiol	9	urine	0,22	0,047	0,023	0,28	192	1,30	70
		stools	0,37	0,38	0,27	1,02	57		
$CaNa_2$ EDTA	9	urine	0,27	0,087	0,03	0,38	258	2,21	113
		stools	0,84	0,36	0,63	1,83	100		
$CaNa_3$ DTPA	9	urine	0,32	0,10	0,003	0,42	285	1,61	82
		stools	0,33	0,30	0,56	1,19	66		
$CaNa_2$ DESTA	9	urine	0,10	0,017	0,02	0,137	100	1,69	83
		stools	0,78	0,29	0,49	1,56	87		
$CaNa_2$ DEETA	9	urine	0,28	0,05	0,01	0,34	235	1,41	73
		stools	0,36	0,28	0,43	1,07	60		
Phosphicine	9	urine	0,38	0,04	0,01	0,43	285	1,72	83
		stools	0,43	0,32	0,54	1,29	70		

TABLE 2. Excretion of Depot Fe^{59} (in % of Amount Administered and of Control)

Complexon administered	No. of animals	Substrate	1st day	2nd day	3rd day	4th day	5th day	During 5th day: as % of amount administered	During 5th day: as % of control	During 5th day: total in urine and stools: as % of amount admin.	During 5th day: total in urine and stools: as % of control
Control	9	urine	0,001	0,001	0,001	0,002	0,003	0,008	100	0,433	100
		stools	0,12	0,13	0,035	0,04	0,10	0,425	100		
$CaNa_2$ EDTA	9	urine	0,007	0,02	0,07	0,1	0,05	0,247	3087	0,977	225
		stools	0,12	0,20	0,15	0,06	0,20	0,730	150		
$CaNa_3$ DTPA	9	urine	0,005	0,02	0,043	0,03	0,01	0,105	1312	0,715	162
		stools	0,22	0,08	0,12	0,09	0,10	0,610	140		
$CaNa_2$ DEETA	9	urine	0,03	0,011	0,053	0,001	0,013	0,108	1315	0,648	126
		stools	0,10	0,06	0,14	0,17	0,07	0,540	127		
$CaNa_2$ DESTA	9	urine	0,05	0,02	0,05	0,05	0,03	0,200	2500	0,840	196
		stools	0,10	0,11	0,16	0,06	0,21	0,640	145		
Phosphicine	9	urine	0,03	0,02	0,03	0,013	0,14	0,107	1315	0,457	105
		stools	0,08	0,09	0,03	0,06	0,09	0,350	82		

increase in the content of iron in the urine. The highest iron concentration in the urine was observed after administration of $CaNa_3$ DTPA and phosphicine: the radioactivity of the urine was increased 16-19 times. The iron concentration in the urine was also raised substantially after administration of $CaNa_2$ DEETA and $CaNa_2$ EDTA.

The action of unithiol and $CaNa_2$ DESTA – the sulfur-containing homolog of $CaNa_2$ DEETA – was weaker. However, this increase was observed immediately after administration of the complexon, and during the following days the iron concentration in the urine fell sharply. On the third day of the investigation less iron was found in

TABLE 3. Effect of Complexons on the Fe^{59} Content in the Tissues

Complexon administered	No. of animals	Blood		Liver		Kidneys	
		% of amount admin.	% of control	% of amount admin.	% of control	% of amount admin.	% of control
Control	6	1,97	100	2,01	100	0,33	100
$CaNa_2$ EDTA	6	3,17	167	1,66	83	0,37	115
$CaNa_3$ DTPA	6	2,07	105	1,70	85	0,39	116
$CaNa_2$ DEETA	6	2,03	103	1,95	97	0,43	130
$CaNa_2$ DESTA	6	2,94	150	2,49	124	0,34	100
Phosphicine	6	2,05	104	1,63	81	0,34	100

the urine of all the experimental animals than of the controls. The more effective the complexon in relation to iron, the less iron was found in the urine after administration of the preparation ceased. For this reason the total amount of iron excreted by the kidneys under the influence of complexons exceeded the iron content in the control animals by only a few times. For instance, in the case of administration of $CaNa_3$ DTPA and phosphicine, the total iron content during 3 days of the investigation was 2.8 times greater than in the controls, with $CaNa_2$ EDTA it was 2.6 times greater, and with DESTA it was 2.3 times greater than the control level, and so on. Administration of the complexons affected not only the excretion of iron by the kidneys, but also the excretory function of the intestine. Whereas in the controls between 10 and 40 times as much iron was excreted by the intestine as by the kidneys, the first dose of complexon caused a sharp modification of this ratio.

A result of this "inversion" of the pathways of excretion was that the administration of complexons did not lead to a significant increase in the elimination of iron in these particular experimental conditions, but, on the contrary, most of the complexons caused a decrease in the excretion of iron to 80-70% of the control level. A slight increase in the excretion of iron was observed when $CaNa_2$ EDTA was used.

Hence the complexons caused no increase in the excretion of iron not stored in depots, but merely changed the pathways of its excretion.

In the next series of experiments the effect of complexons on the fate of the depot iron was studied (Table 2). As in the first series, in series II all the complexons caused a marked increase in the excretion of iron in the urine, but marked differences in the dynamics of its excretion were seen.

Whereas the nondepot iron was sensitive to the influence of complexons during the first day after injection, the first injection had much less effect on the depot iron than subsequent injections. For example, administration of $CaNa_2$ EDTA and $CaNa_3$ DTPA raised the radioactivity of the urine during the 1st day by only 5-7 times, compared with 70-50 and 30-40 times on the 3rd and 4th days respectively. The total amount of iron excreted in the urine after administration of these complexons rose 13-30 times during the time of the investigation. The excretion of iron by the kidneys also rose considerably after administration of $CaNa_2$ DESTA, phosphicine, and $CaNa_2$ DEETA.

It is interesting to note that the deposition of iron in the body led to the almost total cessation of its excretion by the kidneys, whereas the excretion by the intestine remained relatively high. The ratio between the amounts of iron excreted by the kidneys and by the intestine in the control animals ranged from 1:30 to 1:130.

The excretion of the depot iron by the intestine was not reduced by administration of complexons, and in some cases it actually increased. The exception was phosphicine, which caused a slight fall in the iron level in the stools.

The total amount of iron excreted by the kidneys and intestine under the influence of the complexons was increased. The greatest increase was observed after administration of $CaNa_2$ EDTA and $CaNa_2$ DESTA (225 and 196% of the control level respectively). The ratio between the levels of iron in the urine and stools was changed very considerably in every case after administration of the complexons.

The investigation of the effect of complexons on the iron content in the blood and in the principal depots – the liver and kidneys – was of considerable interest (Table 3). The greatest changes took place in the blood. The radioactivity of the blood of the control animals was 1.97% of the injected activity. Complexons causing the excretion of a large amount of Fe^{59}, namely $CaNa_2$ EDTA and $CaNa_2$ DESTA, led to a significant increase in the iron content in the blood. In the liver, forming an iron depot, its level fell, while in the kidneys no change in the radioactivity of the tissue was found.

These results show that complexons have a marked effect on the iron metabolism in the body. This effect is seen primarily in a change in the pathways of excretion of iron: the fraction of iron excreted by the intestine is reduced and the fraction excreted by the kidneys is increased. Iron existing in the body in labile combination with the plasma proteins is eliminated from the body in amounts ranging from 1.3 to 2.21% of the injected dose. The iron present in depots is excreted to a relatively smaller degree (from 0.433 to 0.977% during the period of investigation). This difference between the excretion of depot and nondepot iron is seen especially clearly when its amounts in the urine and stools are compared after the first injection of the complexon. In the first case, despite the large absolute quantities of iron excreted, it is mainly the route of excretion that is changed, but in the second case it is the true excretion. The excretion of Fe^{59} is always increased if the excretion of iron by the intestine does not decrease.

It is clear from the foregoing account that a fuller idea of the metabolism of iron and other metals in normal conditions and during complexon administration can be obtained by studying the excretion of the metals not only by the kidneys, but also by the intestine.

As the results of the investigation showed, the concentration of iron in the blood was increased while that in the liver fell. These two processes are evidently interconnected and the increase in the iron concentration in the blood is a result of its mobilization from its depot in the liver.

SUMMARY

Experiments on albino rats were used to study the influence of $CaNa_2$ salts of ethylendiaminetetraacetic acid and $CaNa_3$ salts of diethylentriaminepentaacetic acid, as well as $CaNa_2$ salts of 2,2'-diaminodiethyl ester of tetraacetic acid and its sulphur-containing analogue, Na_2 ethylenediamine-bis-isoprpylphosphinic acid and sodium dimercaptopropansulphonate on the excretion and distribution of deposited and non-deposited Fe^{59}. The complexons were injected intraperitoneally in doses of 30 mg per rat during 3 days.

All complexons changed the iron metabolism, causing an increase in the iron excretion of the kidneys and a decrease in that of the intestine. The deposited iron was excreted not only by the kidneys but also by the intestine. The largest increase in iron excretion was caused by $CaNa_2$ EDTA and $CaNa_2$ DESTA.

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