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SHORT-TERM TOXICITY OF STRONTIUM CHLORIDE IN RATS

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SUMMARY

A range-finding experiment with strontium chloride hexahydrate (0, 3, 30, 300 and 3000 ppm in the diet) and subsequently a 90-day test with the same compound at dose levels of 0, 75, 300, 1200 and 4800 ppm in a semi-purified diet was carried out with SPF-derived Wistar-rats. The diet contained adequate levels of Ca, Mg, P and Vit.D_3. Growth, food intake, behaviour and mortality were measured, extensive haematology and clinical biochemistry carried out, organ weights determined, X-ray photographs of the bones taken and complete histopathological examination was performed. In addition Sr-content of blood, bone and muscles was determined. Thyroid weights were significantly increased in the males of the 1200 and 4800 ppm group. Histological evidence for increased thyroid activity was noticed in the males of the 4800 ppm group. Pituitary weights were significantly decreased in the females of the 300 ppm and 4800 ppm group, but not of the 1200 ppm group. A histologically confirmed glycogen depletion of the liver was noted biochemically in the highest dose group (4800 ppm).

Sr-content in bone was increased at all dose levels having a constant level from 4 weeks onwards, thus indicating that a no effect level cannot be established. If the increased Sr-concentration in the bone can be considered a non-toxic effect, the non-toxic effect level appears to be 300 ppm.

Abbreviations: AH, aniline hydroxylase; Alk. Pase, alkaline phosphatase; APDM, aminopyrine demethylase; Hb, haemoglobin content; Ht, haematocrit; MCH, mean cellular Hb; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; SGPT, alanine aminotransferase (serum glutamine pyruvic acid transaminase).

INTRODUCTION

Since information on the toxicity of strontium is scarce, it was felt necessary to investigate the short-term toxicity of strontium chloride hexahydrate, a compound used in cosmetics in some countries.

Strontium seems to be a relatively low toxic element [1,3,14,24]. The strontium ion has been found in animal tissues in amounts of 0.01–1 ppm, whereas in bone tissue the Sr-content was found to be as high as 30—80 ppm [1,11].

The oral LD_{50} of strontium chloride hexahydrate ($SrCl_26H_2O$) in the rat is not known, but the lowest published lethal dose of $SrCl_26H_2O$ by intravenous administration is 425 mg/kg body weight [1,14]. In mice the intravenous LD_{50} was reported to be 148 mg/kg body weight [30].

The accumulation of strontium is related with the calcium content in the diet and especially with the mode of administration of strontium [7,19]. The absorption of strontium in the gastrointestinal tract is normally poor [1,31]. The excretion of strontium following oral administration is mainly by the faeces, while after intravenous injection small amounts are excreted by the urine [1,31]. Under certain conditions strontium may induce rickets [4,7,17,26,29].

Strontium in small amounts is essential for growth, especially for the calcification of bones and teeth [1].

MATERIAL AND METHODS

Materials

Strontium chloride hexahydrate ($SrCl_26H_2O$) was obtained from Mallinckrodt, St. Louis, Missouri, U.S.A. Barium content was less than 0.02%.

Animals and diets

SPF Wistar rats came from the breeding stock of the National Institute of Public Health and were housed under conventional conditions, littermate-divided in wire cages, two in a cage according to sex. Tap water and a semi-purified diet (Muracon SSP-tox, Trouw Ltd., Putten, The Netherlands) were given ad libitum. The diet contained 0.05% Mg, 0.75% P, 0.85% Ca and 1.8 I.U. Vit.D_3 per gram.

Statistics

For the purpose of objective quantification of observed differences between separate treatment groups and corresponding controls, Student's *t*-test was used. The results of these testings are indicated as follows: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. According to general statistical theory it is advisable not to attach too much weight to a formal significance result (i.e. $P < 0.05$). In such cases of marginal statistical

significance it is important to consider carefully the test results obtained for corresponding comparisons regarding the other dose levels.

Methods

Determination of strontium in bone, muscle and blood by X-ray spectrometry. Freeze-dried bone samples were washed in a muffle furnace at 700°C for 4 h. After pulverization 0.1 or 0.2 g of the ashed material was mixed with a binding agent (X-ray mix powder, Chemplex Industries, Scarsdale, N.Y.), and pressed into 2 g pellets at a pressure of 20 000 kg. Freeze-dried muscle tissue was weighed, and ashed in activated oxygen in a low temperature asher. The ash was mixed with 2 g of binding agent and pellets were pressed.

0.2 g freeze-dried blood was mixed with 1.8 g binding agent and pellets were pressed.

The pellets were analysed in a Philips PW 1410 X-ray spectrometer, in vacuum, using an X-ray tube with molybdenum anode operated at 60 kV and 40 mA, a scintillation counter, and a lithium fluoride crystal. X-Ray intensity was measured for 40 sec at angles (2θ) of 25.12° (Sr-K α peak), 24.52° and 25.72° (background).

Calibration specimens for the analysis of muscles and blood were prepared by adding known amounts of strontium carbonate to X-ray mix powder and pressing 2 g pellets. As the X-ray intensity is influenced by the presence of bone material in the specimen, calibration specimens for bone analysis were prepared by addition of 0.1 resp. 0.2 g ashed bone materials of the rat and known amounts of strontium carbonate to 1.9 resp. 1.8 g X-ray mix powder.

Sr-intensities in the samples were measured and Sr-concentrations were calculated according to the calibration lines. The limit of detection in the original tissue was 1 mg/kg.

Determination of magnesium and calcium in serum by atomic absorption spectrometry. 100 µl of rat serum was added to 10 ml of an aqueous solution containing 5 g of $SrCl_26H_2O$ per liter. The solutions were analysed, using a Varian Techtron AAS atomic absorption spectrophotometer equipped with Mg and Ca hollow-cathode lamps and a nitrous oxide-acetylene flame. Ca and Mg absorbances were measured at 422.7 nm and 285.2 nm respectively and were compared with calibration lines, prepared with standard Mg and Ca solutions containing the same amount of $SrCl_2$. Sr was added to avoid interferences.

Biochemical methods. SGPT and Alk. Pase were determined with an LKB Reactor Rate Analyzer using Baker Diagnostic Reagents Kits. The microsomal liver enzymes were determined according to the method described by Den Tonkelaar and Van Esch [8]. While glycogen in the liver was measured according to the enzymatic method of Roehrig and Allred [23] using a Boehringer GoD-Perid kit for glucose determination, described

by Fiske and Subbarow [10], a Baker Diagnostic Reagents Kit was used for recording the urea content in the serum.

Range-finding experiment

Rats with an initial body weight of 130—170 g were divided into 5 groups each consisting of 3 females and 3 males, receiving 0, 3, 30, 300 and 3000 ppm $SrCl_26H_2O$ in the diets respectively during two weeks.

The animals were weighed at the beginning of the experiment and after 1 and 2 weeks. Food intake was recorded during the experimental period and was measured per cage (two rats) and expressed as the average intake per day per rat. Food conversion was calculated, blood samples were taken at the end of the experiment and hematological investigation was restricted to Hb, Ht and the number of erythrocytes and leucocytes. The MCV, MCHC and MCH were calculated.

At the end of the 2 weeks X-ray photographs were made of all animals. Strontium concentration was measured in blood, bone and muscle. Liver and kidneys were weighed and examined histopathologically by preparing paraffin sections (5 μm) stained with haemalum and eosin.

90-day experiment

Five groups, each consisting of 10 females and 10 males (body weight 40—60 g) were fed 0.75, 300, 1200 or 4800 ppm $SrCl_26H_2O$ in the diet for 90 days. An additional number of 15 males in the control group and 10 males in the other groups were used for the determination of the Sr-levels in blood, bone and muscle in weeks 0, 4, 8 and 12.

The animals were observed daily and weighed weekly and the food intake was measured in week 2, 5, 9 and 12. Food efficiency was calculated. Haematological investigations were carried out on 10 female and 10 male rats of each group after 90 weeks. The same parameters were studied as was done in the range-finding experiment. In addition the blood picture was studied.

Strontium analysis in blood, bone and muscle and Ca, Mg and P analysis in blood were done in 5 control males in the beginning of the experiment and in 5 males per group after 4, 8 and 12 weeks. In the ninth and the twelfth week of the experiment X-ray photographs were prepared from 2 females and 2 males of group 1 and from 5 females and 5 males of group 5.

After 6 and 12 weeks the activity of SGPT, Alk. Pase and urea content were determined in the serum of 5 males per group. The activities of the microsomal liver enzymes AH, and APDM were determined in 5 males per group after 4 and 12 weeks. The glycogen content in the liver was determined in 5 males after 8 weeks and in 6 females and 6 males after 12 weeks.

After 12 weeks urinalysis was carried out with Bililabstix® (Ames Cy.) in 10 female and 10 male rats. The presence of protein, blood, bilirubin and ketones and also the pH were studied.

After 12 weeks the remaining animals were killed and the weight of brain, pituitary, heart, thyroid, liver, kidneys, spleen, adrenals, ovaries or testes,

uterus or prostate was determined.

Histopathology of these organs and also of lungs, thymus, pancreas, mesenterial lymph nodes, stomach, duodenum, ileum, jejunum, coecum, colon, rectum, urinary bladder, nervus ischiadicus, musculus quadriceps and femur was carried out by preparing paraffin sections (5 μm) stained by haemalum and eosin.

RESULTS

Range-finding experiment

Behaviour, growth, food intake and food efficiency were not affected in the range-finding experiment. Haematological investigation revealed only a slight elevation of the total number of erythrocytes in males and females and a slight increase of the white cell count in the males at the highest dose level. No differences were found in liver and kidney weights and histopathological examination revealed no abnormalities. Sr in blood and muscle were only noted at the highest dose level whereas from 300 ppm onwards increased concentrations were found in bone (Table I).

90-day test

In the 90-day test behaviour, growth, food intake and food efficiency were not affected. After more than 11 weeks one control female died during bleeding procedure.

The slightly elevated erythrocyte count noticed in the range-finding experiment was not confirmed in the 90-day study. A lower leucocyte count noticed in the males of the 300 ppm group is not considered to be caused by

TABLE I

STRONTIUM ANALYSIS OF BLOOD, MUSCLE AND BONE TISSUE IN 3 FEMALE AND 3 MALE RATS PER GROUP AFTER 2 WEEKS (RANGE-FINDING EXPERIMENT)

$SrCl_2$ in diet (ppm)		Sr-content in μg/g wet tissue		
		Blood	Muscle	Bone
Females	0	<1	<1	43 ± 8
	3	<1	<1	49 ± 4
	30	<1	<1	53 ± 5
	300	<1	<1	266 ± 42
	3000	2 ± 0	1 ± 1	1451 ± 127
Males	0	<1	<1	28 ± 7
	3	<1	<1	36 ± 4
	30	<1	<1	52 ± 7
	300	<1	<1	232 ± 15
	3000	2 ± 1	4 ± 3	1711 ± 326

TABLE II

GLYCOGEN CONTENT IN mg/g LIVER IN 3 OR 6 RATS PER GROUP AFTER 8 AND 12 WEEKS (90-DAY EXPERIMENT)

$SrCl_2$ in ppm	0	75	300	1200	4800
Males					
after 8 weeks	31.0 ± 8.8 (3)	21.2 ± 9.2 (3)	32.0 ± 1.9 (4)	23.5 ± 4.7 (3)	32.0 ± 3.8 (3)
after 12 weeks	17.7 ± 6.4 (6)	22.3 ± 6.2 (6)	19.3 ± 6.3 (6)	12.4 ± 4.7 (6)	10.0 ± 6.4 (6)
Females					
after 12 weeks	22.4 ± 6.1 (6)	19.9 ± 7.5 (6)	17.1 ± 3.1 (6)	15.2 ± 5.6 (6)	7.8 ± 3.7 *** (6)

*** $P < 0.001$.

TABLE III

MEAN ORGAN WEIGHT/BODY WEIGHT RATIO IN PERCENTAGES OF 10 FEMALE AND 10 MALE RATS PER GROUP (90-DAY EXPERIMENT)

$SrCl_2$ in ppm	0	75	300	1200	4800
Females					
Brain	0.820 (9)	0.832	0.831	0.843	0.808
Heart	0.321 (9)	0.330	0.324	0.328	0.332
Liver	3.80 (9)	3.71	3.57	3.62	3.57
Kidneys	0.882 (9)	0.914	0.875	0.899	0.948
Spleen	0.230 (9)	0.258	0.235	0.237	0.245
Adrenals	0.0217 (9)	0.0217	0.0201	0.0219	0.0202
Thyroid	0.0071 (9)	0.0082	0.0080	0.0087	0.0074
Pituitary	0.0074 (9)	0.0064	0.0062 *	0.0066	0.0056 **
Uterus	0.182 (9)	0.176	0.164	0.188	0.178
Ovaries	0.0281 (9)	0.0264	0.0256	0.0252	0.0226
Males					
Brain	0.533	0.504	0.538	0.518	0.515
Heart	0.269	0.266	0.271	0.276	0.278
Liver	3.74	3.58	3.64	3.66	3.76
Kidneys	0.751	0.742	0.760	0.736	0.771
Spleen	0.191	0.200	0.189	0.196	0.199
Adrenals	0.0122	0.0119	0.0122	0.0124	0.0128
Thyroid	0.0054	0.0066	0.0064	0.0072 **	0.0068 ***
Pituitary	0.0032	0.0029 *	0.0033	0.0030	0.0032
Testes	0.805	0.773	0.843	0.827	0.795
Prostate	0.128	0.092 **	0.106	0.101 *	0.112

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
Between brackets the number of animals deviating from 10.

TABLE IV

BLIND SEMIQUANTITATIVE EXAMINATION OF LIVER AND THYROID (90-DAY EXPERIMENT)

$SrCl_2$ in ppm	0		75		300		1200		4800	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
LIVER examined:	10	10	10	10	10	10	10	10	10	10
Amount of glycogen in the periphery of the lobule:										
Very little	2	3	3	2	2	1	2	2	4	7
Decreased	3	2	4	4	3	5	4	4	4	
Normal	5	5	3	4	5	4	4	4	2	3
Amount of glycogen in the center of the lobule:										
Very little				1				2	2	2
Decreased	1	5	4	3	2	2	3	3	6	5
Normal	9	5	6	6	8	8	7	5	2	3
THYROID examined:	10	10	9	10	10	10	10	9	10	10
Not activated	5	1	3	3	1	2	4	1	3	2
Very slight activation	3	6	2	3	5	4	4	5	5	3
Slight activation	2	3	3	3	4	4	2	3	2	3
Moderate activation			1	1						2

the treatment, since at higher dose levels such an effect was not observed.

Analysis of Alk. Pase, SGPT and urea in serum did not reveal any significant changes although in the highest dose group an indication of an increased activity of Alk. Pase was noticed. Microsomal liver enzyme activities did not show any changes. The glycogen concentration in the liver after 12 weeks showed a dose-related decrease, which was only significant in the females at the highest dose level (Table II). Urinalysis did not show any abnormalities. The levels of Ca, Mg and P in the blood were not changed at any dose level, and the Ca/P ratio remained constant. The Ca, Mg and P concentrations in blood in all dose groups were higher after 8 weeks than after 12 weeks, which seems a physiological condition.

A significant increase of the relative thyroid weights was found for the males at the two highest dose levels (Table III). Relative pituitary weights of the females at 300 ppm and at 4800 ppm were significantly decreased.

The relative prostate weights were significantly decreased at 75 and 1200 ppm. This criterion, however, must be considered with care, because proper preparation of the rat prostate is difficult.

Neither after 9 nor after 12 weeks changes could be noticed in the X-ray photographs of the animals. Histopathological examination revealed slight

TABLE V

STRONTIUM ANALYSIS OF BLOOD, MUSCLE AND BONE TISSUE IN 5 MALE RATS PER GROUP AFTER 0, 4, 8 AND 12 WEEKS (90-DAY EXPERIMENT)

	$SrCl_2$ in ppm	Sr-content in µg/g wet tissue after week			
		0	4	8	12
Blood	0	<1	<1	<1	<1
	75		<1	<1	<1
	300		<1	<1	<1
	1200		<1	<1	<1
	4800		3 ± 1	3 ± 1	2 ± 1
Muscle	0	<1	<1	<1	<1
	75		<1	<1	<1
	300		<1	<1	<1
	1200		<1	<1	<1
	4800		2 ± 0	2 ± 1	2 ± 0
Bone	0	35 ± 2	20 ± 17	10 ± 4	9 ± 4
	75		276 ± 30	240 ± 19	273 ± 49
	300		619 ± 64	463 ± 76	523 ± 87
	1200		1473 ± 107	1237 ± 103	1430 ± 100
	4800		6088 ± 267	5259 ± 486	5941 ± 783

changes in the liver and thyroid after blind examination (Table IV). The changes consisted of a loss of glycogen in the liver at the highest dose level and a slightly increased activity in the thyroid of the males of the highest dose group.

Again detectable amounts of Sr in blood and muscle were only noticed at the highest dose level whereas the Sr concentration in bone was elevated at all dose levels (Table V).

DISCISSION

In the range-finding test and 90-day experiment of strontium chloride only a few alterations were observed. In the range-finding test a slight elevation of the total number of erythrocytes was noticed, which, however, was not confirmed in the 90-day test. In the 90-day experiment the registered changes were an increased relative thyroid weight in the males of the two highest dose levels and decreased relative pituitary weights in the 300 ppm and 4800 ppm group in females. The increased weight of the thyroid was confirmed histopathologically in the highest dose level. A glycogen depletion in the liver was noticed in the females at the highest dose level by biochemical and histopathological examination.

Strontium analysis in the range-finding test revealed an increase of strontium content in bone in the 300 and 3000 ppm group. In blood and

muscle only marginal levels of detectable Sr were present at the highest dose level (3000 ppm). The same marginal levels were noticed in blood and muscle at the highest dose level in the 90-day test, whereas in bone a dose-dependent increase in strontium content was found.

In evaluating the changes mentioned above, it is important to pay attention to the composition of the diet. Especially the amounts of Ca, Mg, P and Vit.D_3 are important since deficiency of one of these factors may cause rachitic changes in the bone and alterations of the Ca- or P-concentration in blood, bone or muscle [5,7,9,12,13].

In metabolism Sr and Ca are closely related, in that these two elements may replace each other. However, for replacement of Ca in the bone, in such amounts that rickets occurs, the strontium concentration in the blood has to be considerably higher than the Ca concentration [25]. Calcium and strontium are not transported in the same way: relatively more Ca is bound to the serum proteins [16]. Moreover it is known that Sr is absorbed at a much lower rate than Ca [1].

Magnesium, phosphate and Vit.D_3 are also important factors which may indirectly change calcium metabolism [15,20—22].

Strontium may inhibit the Ca-absorption by interfering the conversion of 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol, thus leading to a decreased production of Ca-binding proteins [17].

A sufficiently high level of Sr in the serum may lead to a change in the Ca/Sr ratio in the body which can cause a partial replacement of Ca by Sr in the serum binding proteins and to a netto Ca secretion in the small intestine [27].

In the present experiment, however, the results indicate that although an increased strontium concentration in bone is already found after 4 weeks in the 75 ppm $SrCl_2$ dose group, the calcium concentration in the blood remains unchanged in all dose levels. In addition the Mg and P concentrations in the serum are unchanged whereas also the Ca/P ratio is constant. Moreover, rachitic changes as described by Storey (1962) could neither be found in X-ray photographs nor after histopathological examination.

The dose levels in the present study, however, were lower (1600 ppm Sr) than those which could induce rachitic changes (6000 resp. 5896 ppm Sr) [7,26].

The increased thyroid weights confirm earlier findings of Filer et al., 1966, who found a weight gain and hypertrophy of thyroid gland after the administration of strontium to the miniature swine. They additionally found an increased weight of the adrenals and kidneys, which however, was not observed in the present experiment. Eger and Laup [6] noticed histologically an activation of the thyroid of man. Thyroid hypertrophy may be indirectly induced by Sr through activation of thyrocalcitonin production. This however seems unlikely since no parafollicular cell hyperplasia was noticed and Ca levels in the serum were normal.

Filer et al. [9] state that thyroid hypertrophy may be due to a calcium

deficiency in relation to the dietary iodine content. In the present study however both Ca (0.85%) and iodine levels were sufficient. Therefore this phenomenon needs further investigation.

The decreased pituitary weights seem not to be dose related and are difficult to interpret.

The reduced weight of the pituitary in females has not been described, the only effect known in the literature on the pituitary is the role of strontium in the potassium stimulating effect on the posterior pituitary, resulting in a release of neuropeptid hormones [2].

The glycogen depletion noticed in this experiment may be caused by several factors other than strontium, such as stress, starvation or diurnal rhythm.

The glycogen depletion was only significantly decreased at the highest dose level.

The strontium concentrations in the bone in the 90-day experiment were increased at each dose level. It was observed that the Sr-concentrations reached already a constant level after 4 weeks. This indicates that at a certain dosage a steady state may be derived [6,18]. Of interest is the decrease in time of the Sr-content in the bones of the control animals, the reason of which is not yet understood.

If the findings above are summarized it is evident that a "no determined effect level" can not be established if an increased Sr concentration in bone is considered to be an effect.

It might be of interest to investigate at what level of Sr concentration in the bone the first signs of rickets occur. In this way information can be obtained about the differences in Sr levels in the bone under rachitic conditions compared to the levels noticed when 75 or 300 ppm of $SrCl6H_2O$ is given in the diet. This question is partially answered by the work of Schmid and Gutschow [26]. In Sr-induced rachitic bones they found Sr-levels of 7—8% in the ash-residue. In the present study the Sr-levels in the ash-residue of bone were respectively approx. 0.005, 0.06, 0.12, 0.35 and 1.6% in the different groups (0.75, 300, 1200 and 4800 ppm). This means that at the 75 or 300 ppm dose level the Sr-concentration is around 100 or 50 fold less than in rachitic conditions. If the increased Sr-concentration in the bone can be considered as a non toxic effect, the "no determined toxic effect level" appears to be 300 ppm since at the 1200 and 4800 ppm level significant changes in thyroid weights were found.

REFERENCES

1 E. Browning, Toxicity of Industrial Metals, Butterworth, London, 1969, pp. 302—306.
2 M. Buchs, J.J. Dreifuss, J.D. Grau and J.J. Nordmann, Proc. Physiol. Soc., 222 (1972) 168.
3 V.V. Cole, B. Harned and R. Hofkesbring, J. Pharm. Exp. Ther., 1 (1941) 71.
4 L.B. Colvin, C.R. Creger, T.M. Ferguson and H.R. Crookshank, Poultry Sci., 51 (1972) 576.

5 R.A. Corradino, J.G. Ebel, P.H. Craig, A.N. Taylor and R.H. Wasserman, Calc. Tiss. Res., 7 (1971) 93.
6 W. Eger and H. Laup, Beitr. Path. Anat., 113 (1952) 337.
7 B. Engfeldt and S.O. Hjertquist, Arch. Pathol. Abt. A, 346 (1969) 330.
8 E.M. den Tonkelaar and G.J. van Esch, Toxicology, 2 (1974) 371.
9 L.J. Filer Jr., H. Churella, R. Knauff and O.W. Vaughan, in Proc. Symp. Swine Biomed. Res., Richland, Washington, 1966, pp. 151—162.
10 C.H. Fiske and Y. Subbarow, J. Biol. Chem., 66 (1925) 375.
11 R.M. Forbes, H.H. Mitchell and A.R. Cooper, J. Biol. Chem., 223 (1956) 969.
12 D.R. Fraser and E. Kodicek, Nature New Biol., 241 (1973) 163.
13 L. Galante, K.W. Colston, J.M.A. Evans, P.G.H. Byfield, E.W. Malthaus and J. McIntyre, Nature, 244 (1973) 438.
14 D. Loeser and A.L. Konwiser, J. Lab. Clin. Med., 15 (1929) 35.
15 S. Nordio, A. Donath, F. Macagno and R. Gatti, Acta Paediat. Scand. 60 (1971) 449.
16 P.J. Nijweide, Proc. Kon. Ned. Acad. v. Wetens., 77 (1974) 367.
17 J.L. Omdahl and H.F. de Luca, J. Biol. Chem., 247 (1972) 5520.
18 R.F. Palmer and R.C. Thompson, Am. J. Physiol., 207 (1964) 561.
19 C. Pecher, Proc. Soc. Exp. Biol. Med., 48 (1941) 86.
20 H. Rasmussen, M. Wong, D. Bikle and D.B.P. Goodman, J. Clin. Invest., 51 (1972) 2502.
21 C.R. Reddy, J.W. Coburn, D.L. Hartenbower, R.M. Friedler, A.S. Brickman, S.G. Massry and J. Jowsey, J. Clin. Invest., 52 (1973) 3000.
22 C.R. Reddy and B. Sivakumar, Lancet, 1 (1974) 963.
23 K.L. Roehrig and J.B. Allred, Anal. Biochem., 58 (1974) 414.
24 C.H. Rogers, T.O. Soine and C.O. Wilson, A Textbook of Inorganic Pharmaceutical Chemistry, Lea and Febiger, Philadelphia, 1952, pp. 449—450.
25 A. Schmid, Z. Physiol. Chem., 326 (1961) 177.
26 A. Schmid and K. Gutschow, Arch. Toxicol., 23 (1968) 245.
27 A. Schmid and G. Kempf, Arch. Pharmakol., 269 (1969) 300.
28 E. Storey, Austral. Ann. Med., 10 (1961) 213.
29 E. Storey, J. Bone J. Surg., B44 (1962) 194.
30 J.B. Syed and H. Fazle, Toxicol. Appl. Pharmacol. 22 (1972) 150.
31 E.J. Underwood, Trace Elements in Human and Animal Nutrition, 3rd ed., Academic Press, New York, 1971, pp. 448—449.