7. No *CX* was detected in cerebrospinal fluid after administration of this substance.

8. The aggregate amount of *CX* found in the urine and faeces and recovered from the animal body after 48 hr. accounted for only approximately 55% of the amount of *CX* given.

9. The absorption of injected *CX* is a slow process and is incomplete even 48 hr. after the injection.

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The Replenishment of Depleted Skeletal Reserves of Magnesium

By J. DUCKWORTH and W. GODDEN, *The Rowett Institute, Aberdeen*

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It has been shown that the Mg of the skeleton is mobilized in response to the threatened shortage of the element in the soft tissues that results from dietary insufficiency of Mg [Duckworth, Godden & Warnock, 1940]. In the face of the persistent demand for Mg in Mg deficiency it was found that the rate of skeletal growth determined the amount that could be liberated on account of the demand of the skeleton itself for Mg [Duckworth & Godden, 1941]. The most striking feature of the mobilization of Mg was the high speed with which large quantities were released.

The question that immediately suggests itself is whether the skeletal stores can be replenished as easily as they are depleted. The problem is of practical interest in the nutrition of farm animals and man, in which the dietary intake of Mg is liable to vary. This is especially true in the case of man for whom vegetables, the supply of which fluctuates throughout the year, are the most important single source of Mg [Duckworth & Warnock, 1942–3]. The result is that varying degrees of inadequacy or alternating periods of adequacy and inadequacy are more common than constant minor degrees of insufficiency. This state may be further accentuated by losses in sweat, which may become abnormal owing to seasonal change in temperature or conditions and intensity of work [Duckworth & Warnock, 1942–3].

The present study was undertaken to examine this problem, and because of the profound influence of the rate of bone growth on liberation of skeletal Mg, a combination of Mg deficiency with different degrees of Ca deficiency was used. This approach also allowed the examination of certain points of interest concerning the interrelation of Ca and Mg in the skeleton.

METHODS

Diets used. The preparation of the diet, the methods of analysis and the management of the animals have been reported [Duckworth *et al.* 1940; Duckworth & Godden, 1941] and need not be repeated here. The technique for preparing a diet low in Mg simultaneously removes the Ca with about the same degree of efficiency, so that no additional procedure is required. The diets contained less than 6 parts per million (p.p.m.) of Mg and about 1–2 p.p.m. of Ca.

Four diets were prepared, each with a P content of 0·35%. By additions of $CaCO_3$, with slight compensatory adjustments in the starch content, these diets were arranged to contain 0·90, 0·30, 0·10 and 0·00% of Ca, and are referred to as diets A, B, C and D respectively.

Animal management. One litter of eight rats (4 ♂ and 4 ♀) at weaning was allotted to each diet. Of each litter six rats were placed on the appropriate deficient diet while two remaining rats of the litter were given the same diet supplemented with sufficient $MgSO_4$ to raise the Mg content to 0·07%. The six animals undergoing skeletal Mg depletion were observed closely, and upon the death of one member of the set another, judged clinically to be close to death, was dosed with several drops of a solution of $MgSO_4$ and then transferred to the same diet supplemented with Mg. Thus of each litter three animals (hereafter referred to as 'depletion rats') died of Mg deficiency and three others (subsequently referred to as 'curative rats') were sacrificed after each had received a curative period of 10 days on the appropriate Mg-supplemented diet. The two remaining animals were treated as controls, one being killed at what was estimated to be the average

time of survival of the three depletion rats and the other being killed 10 days later. These animals are referred to as the 'depletion control' and the 'curative control' respectively.

The animals were under constant supervision during the day and were visited every 4 hr. during the night. Thus differences between the corresponding depletion and curative animals, from the standpoint of length of time on the Mg deficient diets, were minimized.

RESULTS

The average values for bone composition are given in Table 1. Individual values for the percentage of Mg in bone ash are given in Fig. 1.

Table 1. *Composition of bones of rats receiving different types of diets*

Group*	Wt. of bones (mg.)	Wt. of ash in bones (mg.)	Wt. of ash in bones (%)	Wt. of Mg in ash (mg.)	Wt. of Mg in ash (%)	Mg deposited during curative period (mg.)
A —	362	204	56·4	1·14	0·56	
A —+	524	312	59·5	2·46	0·79	1·32
A 1 +	350	201	57·4	1·94	0·97	
A 2 +	544	332	61·0	3·00	0·90	
B —	363	195	53·7	1·10	0·57	
B —+	565	327	57·9	2·51	0·77	1·41
B 1 +	370	207	55·9	2·00	0·97	
B 2 +	642	374	58·3	3·19	0·85	
C —	388	191	49·2	1·06	0·56	
C —+	461	235	51·0	1·95	0·83	0·89
C 1 +	406	208	51·2	2·08	1·00	
C 2 +	448	227	50·7	2·12	0·93	
D —	279	135	48·4	0·76	0·56	
D —+	266	116	43·6	1·08	0·93	0·32
D 1 +	268	122	45·5	1·31	1·07	
D 2 +	320	130	40·6	1·42	1·10	

* — indicates depletion rats; —+ indicates curative rats; 1 + indicates depletion control rats; 2 + indicates curative control rats. Letters indicate the diet used (see 'Methods').

The average survival periods of the depletion rats on Mg-deficient diets A, B, C and D were, respectively, 5·0, 7·3, 8·7 and 11·3 days. Comparison of

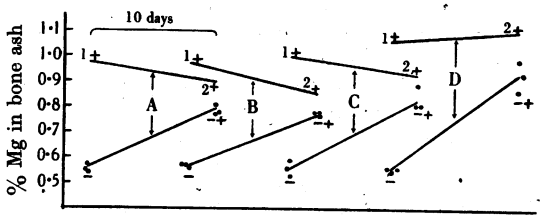

Fig. 1. Percentages of Mg in the bone ash of rats receiving different diets and treatment. — indicates depletion rats; —+ indicates curative rats; 1 + indicates depletion control rats; 2 + indicates curative control rats. Letters indicate the diet used (see 'Methods').

the data for bone growth should be made with these differences in mind. Since a more extensive study has already been published [Duckworth & Godden, 1941] of bone development in rats receiving the present diets, further discussion is unnecessary.

The percentage of Mg in the ash was about the same in all depletion rats irrespective of diet. After 10 days of re-alimentation with Mg the influence of different degrees of Ca adequacy became apparent. Thus the curative rats receiving diets A and B showed closely similar percentages of Mg in the ash, but the curative rats receiving diets C and D showed progressively higher concentrations with decreasing Ca intake. It will be noted that the individual values were not so closely spaced in the two latter groups. The control rats receiving diets A, B and C showed the characteristic reduction of Mg percentage with increasing age. The control rats receiving diet D did not show this reduction and the values were above those of controls receiving the normal diets A and B. There is, perhaps, some indication of increased Mg percentage in the control rats receiving diet C. In none of the curative animals was the value of the corresponding curative control reached, although one rat on diet C and all on diet D approached, or surpassed, the curative control values for diets A and B.

The amounts of Mg in the bone were closely similar for corresponding rats of diets A and B. The curative rats and the curative control of diet C showed, by the lower quantities of Mg and ash, the influence of restricted Ca intake that had not been indicated in the depletion rats and the depletion control, which had received the diet for a shorter period. The amounts of Mg in the bones of rats receiving diet D were lower in all cases than for corresponding rats on other diets.

The quantities of Mg stored by the curative rats, estimated by difference between the values for depletion and curative animals, were about the same in rats of diets A and B. The value was less in animals receiving diet C and least in those receiving diet D.

The symptomatology, including cessation in growth, did not differ from that of previous studies. After the addition of Mg, curative rats of diets A and B grew normally, rats of diet C showed subnormal growth and those of diet D slight increases.

DISCUSSION

The regularity of the individual values for the percentage of Mg in bone ash, given in Fig. 1, indicates that the differences observed in the curative littermates at the time they were killed can be attributed to differences in the degree to which Mg is stored under different dietary conditions. This is supported

by the close similarity in the results observed for the curative rats receiving diets A and B, which are essentially normal diets.

It is well known, and the evidence has been summarized elsewhere [Duckworth, 1938–9], that when demineralization of the skeleton occurs the Mg content of the bone ash generally increases. Because of the proportional increase in organic matter, itself containing Mg, during demineralization, it has not been possible to state categorically that the increase found in the percentage of Mg in the bone ash arose from an increase in the Mg content of the crystals of bone salt. The possibility that the increase was the outcome of a greater amount of organic matrix remained.

The present findings contribute information about this aspect of the interrelation of Ca and Mg in bone. The Mg content of the ash of the control animals increased as the Ca supply diminished, the customary reduction with age giving way to an increase with progressive demineralization in the case of diet D. In the depletion rats this did not happen, irrespective of the degree of calcification of the bones. The curative rats receiving diets C and D showed progressively greater percentages of Mg in the ash as Ca intake fell. The simplest explanation of these changes is that Mg can replace Ca, to a limited extent, in the bone salt crystal during periods of Ca insufficiency. If, however, there is simultaneously a deficiency of both Ca and Mg, then this replacement does not occur. When, after a period of both Ca and Mg lack, the Mg is restored to the diet, it then begins to exert its Ca-sparing effect in the skeleton. It cannot be argued convincingly that the increase in the percentage of Mg in the ash of rats receiving diet D arose in any way from the greater percentage of organic matter. This would imply that the organic matrix of bone contained many times more Mg/unit weight than other tissues [the data are collected in the review of Schmidt & Greenberg, 1935] and that this higher content could be dispensed with in Mg deficiency to give the constant results found for the depletion rats.

Tufts & Greenberg [1937–8*a*] showed that the Mg content of soft tissues is not reduced in Mg deficiency. Thus, when Mg is restored to the diet after a period of deficiency, there is no soft tissue deficit to remedy, and the only requirements are those for the replenishment of the skeletal reserves and for new soft tissue and skeletal growth. Since Tufts & Greenberg [1937–8*b*] obtained good growth, normal gestation and parturition, and fair lactation, in rats whose diet contained only 0·005 % Mg, it is unlikely that the present diet failed to supply sufficient Mg to meet the above demands. This failure of the curative rats to attain the same percentage of Mg in the ash as the corresponding curative control rats may have arisen from either of two main causes, or from a combination of them. The first cause of failure may be that Mg cannot re-enter the depleted crystal, or that it can only re-enter it at a very slow rate. The second may be that new bone salt deposited when Mg is restored to the diet does not initially contain the normal quantity of Mg, normal composition being attained only gradually. That this failure almost certainly involves the former of these alternatives is shown by earlier studies [Duckworth & Godden, 1941], where it was found that the return of plasma Mg to normal levels during the second phase of the deficiency, when drafts on the skeletal Mg deposits have ceased, was not accompanied by a return of Mg to the skeleton. A further opportunity for Mg to enter bone salt would be during those processes of solution and redeposition of bone salt which, although detected, have yet to be measured accurately. This additional process was not considered above when the replenishment of the reserves of the growing animal was discussed, but it may have been mainly responsible for the re-entry of Mg into the bone salt, in the curative rats receiving diet D in which no bone growth occurred.

Irrespective of the manner in which Mg returns to the depleted bone salt it is clear that the reparation of the deficit is less rapid than the depletion process. Attention has been called elsewhere [Duckworth & Warnock, 1942–3] to the wide variations in the concentrations of Mg in human bone, and to the evidence of dietary deficiencies in some social groups presented. It may be that the variation noted is evidence not only of depletion but also of the slow rate at which the deficit is made good when Mg supplies become adequate. It might be expected that in the adult the return of bone composition to normal, in respect of Mg, would require longer than in the young growing animal, because of the absence of active bone growth. In this case, however, the mechanism involved may resemble that of the curative rats of diet D, and may depend on the processes of solution and re-precipitation mentioned above.

SUMMARY

1. Evidence is presented to show that the increase of Mg in bone ash which occurs in demineralization arises from a Ca-replacing action of Mg. It does not occur if Mg supplies are inadequate.

2. The replenishment of skeletal Mg reserves, in rats depleted by acute Mg deficiency, is incomplete after a 10-day period of re-alimentation with Mg, irrespective of whether or not bone growth occurs.

3. The interrelation of Ca and Mg in the skeleton is discussed in the light of the experimental findings

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The Iodometric Micro-determination of Arsenic in Biological Material

By G. A. LEVVY, *Departments of Medical Chemistry and Pharmacology, University of Edinburgh*

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The method of Allcroft & Green [1935; see also Kiese, 1937] for the titrimetric estimation of arsenic in tissue digests yielded satisfactory results with 0·05–1 mg. As. As is separated as AsH_3, which is passed into $AgNO_3$ solution, the arsenite formed being titrated with dilute I_2. It has been found possible to adapt this technique for 5–50 μg. As by reducing the volume of the solution to be titrated till the I_2 required for a distinct colour change is a small fraction of that required to oxidize the arsenite.

Tubes have been constructed in which 1·5 ml. N/50 $AgNO_3$ are sufficient for efficient absorption of AsH_3 at the rapid rate of passage of H_2 necessary for complete separation of As as AsH_3. Titration with N/200 I_2 is carried out in the absorption tube. Under the conditions of the experiment, a distinct colour change is produced by 0·003 ml. I_2, although the amount may vary slightly with the observer and with the lighting. The microburette described by Conway [1939], with a ground-glass joint in place of the rubber stopper in the standard model, is used in the titration. If the reservoir is shielded from light, there is little change in the I_2 over intervals of weeks.

The procedure described has been found reliable in thousands of routine estimations carried out by several workers in these departments and elsewhere. No difficulty has been encountered in adapting it to many types of material.

OUTLINE OF METHOD

Organic matter is destroyed by digestion with a mixture of HNO_3, $HClO_4$ and H_2SO_4. The digest is treated with $(NH_4)_2C_2O_4$ to remove interfering N compounds. When the biological material is rich in As and the sample taken for analysis is small ('Digestion—small scale'), AsH_3 is evolved directly from the digest. With large samples of material ('Digestion—large scale'), As is distilled from the digest as $AsCl_3$, and AsH_3 is evolved from the acid distillate. The procedure thenceforth is the same in both cases. After adjusting the acid concentration and adding $CuSO_4$ and $SnCl_2$ to catalyze H_2 evolution, Zn is introduced into the As-containing solution and the gas liberated is passed through 10N NaOH and N/50 $AgNO_3$. The dimensions of the $AgNO_3$ tube are shown in Fig. 1. To avoid risk of breaking the tip of the Conway burette, the gas inlet (thick-walled capillary tubing of 1 mm. internal diam.)

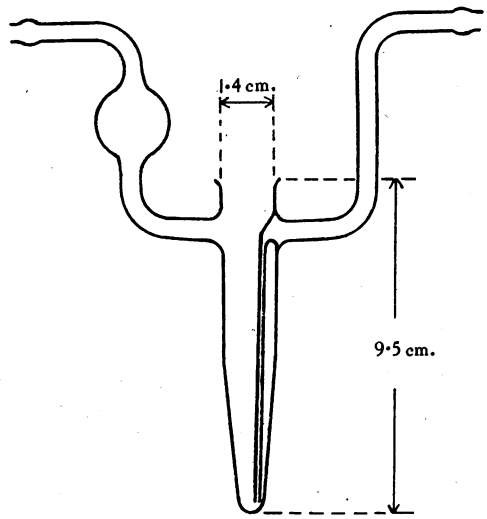

Fig. 1. The absorption tube.

should be situated close to the wall, as shown. Before titrating the arsenite in the absorption tube, solid KI is added to remove excess Ag^+ which would otherwise prevent the appearance of the I_2-starch colour. This is followed by $NaHCO_3$ to remove HI produced in the titration, and starch indicator. The tip of the Conway burette is immersed in the solution, which is stirred during the titration by passing air through the gas inlet of the absorption tube.

Since a large number of digestions can be carried out simultaneously, the time involved per sample at this stage of the determination is negligible. By working in serial fashion with six sets of apparatus, estimations can be put through the stage of AsH_3 separation and arsenite titration at about 10 to the hour. When preliminary separation of As as $AsCl_3$ is necessary, the distillations can be done at the rate of one every 10 min. by using duplicate apparatus.