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## Adequacy of Cow Milk as a Source of Magnesium for Rats.

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Duncan, Huffman and Robinson[1] reported that calves restricted to whole milk diets alone, or supplemented with iron, copper and manganese, eventually manifest tetany which is indistinguishable from tetany in rats on a magnesium-low diet.[2] The tetany was prevented and serum magnesium was restored to normal by the administration of magnesium carbonate or magnesium oxide.[3] Since the content of magnesium in milk appears to be rather low, in relation to the calcium and phosphorus, it is possible that prolonged restriction to milk diets of young of other species might eventuate in magnesium deficiency, as suggested by Schmidt and Greenberg.[4] According to data cited by Cox and Mueller,[5] milk of cows and humans may contain about 0.013 and 0.005% magnesium respectively. Analyses of rat milk gave 0.031% magnesium.[5]

But in several investigations rats have been reared to adulthood on cow milk supplemented with iron and copper and in no instance have symptoms been reported which indicate the occurrence of magnesium deficiency.[6] This suggests that rats are either (a) better able than calves to utilize the magnesium of cow milk or (b) their requirement for the element is less than that of calves. Duncan and associates believed that " . . . . there is a failure in the magnesium metabolism which prevents the animal (calf) from utilizing the available magnesium." Whatever might be the explanation of these observations, the findings suggest that direct investigation should be made of the adequacy of cow milk as a source of magnesium for rats.

Young rats weighing 40 to 45 g were used. They were kept in individual cages and fed exclusively commercial grade A pasteurized cow milk *ad libitum*, supplemented with 0.25 mg thiamin per liter.

1 Duncan, C. W., Huffman, C. H., and Robinson, C. S., *J. Biol. Chem.*, 1935, **108**, 35.

2 Kruse, H. D., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1933, **100**, 603.

3 Huffman, C. H., and Duncan, C. W., *J. Dairy Sci.*, 1936, **19**, 440.

4 Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 297.

5 Cox, W. M., Jr., and Mueller, A. J., *J. Nutr.*, 1937, **13**, 249.

6 Underhill, F. A., Orten, J. M., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932-33, **99**, 469.

Mineral supplements fed to all the rats were Mn, Fe and Cu at daily levels of 3.4, 2.4 and 0.2 mg respectively per rat. These were furnished as $MnSO_4 . 2H_2O$, $FeCl_3 . 6H_2O$ and $CuSO_4 . 5H_2O$. Twelve rats (A), sexes evenly divided, were given this basal ration supplemented with 0.194 g magnesium, as $MgSO_4 . 7H_2O$, per 1000 cc of milk. This increased the content of magnesium approximately 100%, since, by analysis, the milk contained about 0.230 g magnesium per 1000 cc. Twelve other rats (B), similarly selected, were given the same basal ration and enough $H_2SO_4$ to equal the sulfate fed to rats A. After 56 days 4 of the rats in each group were sacrificed. Determinations were made of serum magnesium, bone ash and bone calcium and magnesium, using methods described by Orent, Kruse and McCollum.[7] On the seventy-fourth day the remaining rats were killed and the same determinations were repeated.

No symptoms were observed which suggested that the rats suffered any degree of magnesium deficiency. The average weights of males and females at the end of the study were 196 and 111 g respectively for those given the supplement of magnesium (Group A); whereas the weights of those without the supplement (Group B) were 207 and 112 g, respectively. The average weekly milk consumption of Group A was 375 cc and that of Group B was 370 cc. Marked variation was noted in the milk intake, a few rats ingesting 80 to 90 cc each per day while others took only 30 to 40 cc. The content of serum magnesium was not appreciably affected. The averaged value for Group A was 3.3 mg per 100 cc and that for Group B was 3.1. Similarly the bones showed no evidence of actual magnesium deficiency, although rats given milk fortified with magnesium had a larger percentage of bone magnesium than the unsupplemented controls (Table I).

In constructing Table I no distinction was made between values for rats killed after 56 days and those killed after 74 days, since the

TABLE I.
Calcium and Magnesium Content of Combined Femura, Tibiæ and Fibulæ of Rats on a Milk Diet, with (A) and without (B) Magnesium Supplementation.

| | Bone ash per rat, g | | % of ash | | | |
|---|---|---|---|---|---|---|
| | | | Ca | | Mg | |
| | A | B | A | B | A | B |
| No. of analytical values | 12 | 12 | 12 | 12 | 11 | 10 |
| Mean | .8121 | .8111 | 38.41 | 38.53 | .729 | .678 |
| Standard deviation | .1688 | .1766 | 0.50 | 0.33 | .049 | .039 |

[7] Orent, E. R., Kruse, H. D., and McCollum, E. V., *J. Biol. Chem.*, 1934, **106**, 573.

difference between the two was slight. The average percentage of bone magnesium in Group A rats was 0.714 after 56 days and 0.737 after 74 days. For Group B rats it was 0.676 after 56 days and 0.691 after 74 days, indicating that the content of bone magnesium was not decreasing as restriction to the milk diet continued.

Since, in addition to the normal serum magnesium and absence of deficiency symptoms, the magnesium content of bone did not continually decrease during the experimental period, it is concluded that cow milk contains enough magnesium to prevent magnesium deficiency in rats. This suggests that the magnesium requirement of rats is either (a) lower than that of calves, or (b) rats are better able than calves to utilize the magnesium of cow milk.

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## Anticatalase Activity of Sulfanilamide and Related Compounds. III. Oxygen Tension and Bacteriostasis in Pneumococcal Cultures.

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In previous publications[2-5] the authors have investigated the theory, originally proposed by Locke,[1] that the retardation of growth of certain microörganisms in the presence of sulfanilamide may be primarily the result of the accumulation of hydrogen peroxide. This accumulation is presumed to arise through the inhibition of catalase by sulfanilamide which has been activated by oxidation. It was demonstrated[2] that sulfanilamide and many structurally related compounds have an appreciable anticatalase activity which is frequently enhanced by oxidative processes such as those involved in ultraviolet irradiation and[5] that bacteriostasis of Type I pneumococcus *in vitro* is accompanied by a correspondingly marked accumulation of hydrogen peroxide in the culture.

The formation of hydrogen peroxide requires time and oxygen,

1 Locke, A., Main, E. R., and Mellon, R. R., *Science*, 1938, **88**, 620.

2 Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

3 Locke, A., Main, E. R., and Mellon, R. R., *J. Immunol.*, 1939, **36**, 183.

4 Mellon, R. R., *Modern Hospital*, 1938, **51**, 53.

5 Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.