

CCLV. THE NORMAL SERUM-CALCIUM AND MAGNESIUM OF THE RAT: THEIR RELATION TO SEX AND AGE.

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THE normal serum-magnesium of the rat has not hitherto been determined, except perhaps for a very few animals; some figures given by Watchorn [1932] for instance, were too few to be of value. The present paper gives the data from approximately 100 rats. The opportunity was taken to study the calcium also, for although the rat has frequently been used for serum-calcium studies (notably in work on irradiated ergosterol) the normal has received but little attention, a few animals only having generally been used. The work of Cameron referred to later is, however, an exception to this general statement.

EXPERIMENTAL.

The rats (black and white piebalds) when weaned were placed on a diet consisting of "light white casein" 23, rice starch 40, cane sugar 20, arachis oil 12, salts (modified McCollum and Davis, for details see Watchorn [1932]) 5, dried yeast 10, cod-liver oil 2.5. Fresh water was provided *ad lib.*, and in addition fresh milk was given several times a week. The percentage of magnesium in the basal diet was 0.035, which is within the range suggested by Lavollay [1931] as most suitable for growth. The males and females were kept separate throughout, and all animals were housed in large cages giving ample room for exercise. The room was kept at a constant temperature of 70° F. The rats were always killed in the early afternoon and the stomach was invariably empty, no fresh food having been given that morning. Blood was removed by heart puncture under chloroform anaesthesia. All females were taken in dioestrus, and each rat was examined for evidence of disease; if such was present the blood was not used. Any haemoglobin-stained sera were also discarded.

Calcium was estimated by Clark and Collip's [1925] modification of Kramer and Tisdall's method, and magnesium by the Bell-Doisy colorimetric determination of the precipitated magnesium ammonium phosphate. Owing to the high concentration of magnesium in the serum of the rat it is possible to make accurate determinations on 1 cc. of serum. 2 cc. were, however, taken whenever possible on account of the accompanying calcium determination.

Results.

The rats of each sex have been divided into two groups, "young" and "adult." The former were all 3-4 months old, and the latter ranged from 8½ to 16 months. Originally the adults were subdivided into those under one

year and those over one year, but as no difference could be detected in the few 16-months old rats available, they have finally been grouped together with the others. The results are given in Tables I and II.

About 20 rats reared on a mixed stock diet of natural foodstuffs have also been examined. The values for serum-calcium and magnesium all came within the normal limits given in the following tables.

Table I. *Normal rat serum-calcium.*

	(mg. per 100 cc.)			
	Males		Females	
	Young	Adult	Young	Adult
No. of animals	28	27	24	24
Range of variation	10.70-13.79	10.29-13.16	11.18-13.23	9.61-14.04
Mean value	12.23	11.92	12.00	11.60
Standard deviation of the mean	± 0.14	± 0.16	± 0.12	± 0.26
Standard deviation of the series	0.75	0.84	0.61	1.29

Table II. *Normal rat serum-magnesium.*

	(mg. per 100 cc.)			
	Males		Females	
	Young	Adult	Young	Adult
No. of animals	28	25	23	23
Range of variation	4.01-6.89	3.30-5.24	3.71-5.10	3.63-5.69
Mean value	5.36	4.43	4.39	4.43
Standard deviation of the mean	± 0.12	± 0.10	± 0.08	± 0.10
Standard deviation of the series	0.64	0.49	0.39	0.50

DISCUSSION.

Calcium. From a much smaller series Parhon and Werner [1932] found that the normal serum-calcium of the rat varied from 11.0 to 13.5 mg. per 100 cc., with an average of 12.2 mg. Tweedy and Chandler [1929] give a range of 9.25-12.5 mg. per 100 cc., and Hess *et al.* [1932] in a series of 11 normal young rats have values from 10.1 to 13.0 mg. per 100 cc., the average being 11.18 mg. The figures in Table I are of the same order as those just quoted, as also are those given by Dixon [1933]. On the other hand, Hess *et al.* in an earlier paper [1928-29] state that the rat's normal serum-calcium is 10.0 mg. per 100 cc., and Harris and Stewart [1929] that the range is 9.5-10.5 mg., though in neither case are figures given or authorities quoted. The "hypercalcaemic" figure found by Harris and Stewart seems in fact to be well within the normal range.

In the present series the serum-calcium level of both young and adult females was slightly lower than that of the corresponding males. The adults of both sexes had lower average values than the young. The question arises as to whether these small differences can be regarded as significant. If the animals are grouped into "young" and "adult" irrespective of sex and the results treated statistically, the following figures are obtained:

Mean value of all young	= 12.16 mg. per 100 cc.
Mean value of all adult	= 11.77 mg. per 100 cc.
Difference	= 0.39 mg. per 100 cc.

The value of *t* is 2.171, *P* lies between 0.05 and 0.02, and thus the difference due to age is significant. Parhon and Werner [1932] noticed a tendency for

serum-calcium to decrease with age in many species, including the rat. Cameron [1928] found the contrary.

Males and females grouped irrespectively of age give the following figures:

Mean value of all males	= 12.10 mg. per 100 cc.
Mean value of all females	= 11.80 mg. per 100 cc.
Difference	= 0.30 mg. per 100 cc.

The value of t is 1.708, so that P lies between 0.1 and 0.05; from this it is impossible to say with certainty whether the difference between sexes is significant or not. McIsaac [1928] found slightly lower concentrations of calcium in the serum of young female rabbits compared with males of similar ages; this sex difference became more marked with age. Meglitzky [1927] found a similar sex difference in cats, and Boynton and Greisheimer [1930-31] for men and women. Charles [1931] found an average of 11.96 mg. per 100 cc. for 12 male rabbits and 11.51 mg. for 12 females—though again, statistically, the difference is not significant.

Boynton and Greisheimer [1930-31] noticed that men showed a smaller range of serum-calcium variation than women, and Okey *et al.* [1930] state that the day to day variations for men are smaller than for women. It is apparent from Table I that the serum-calcium of the adult female rat is more variable than that of the adult male or the young of either sex.

Cameron and Williamson [1927] give the average winter and summer serum-calcium value for the albino rat as 10.4 mg. per 100 cc., with a range of 8.3-12.1 mg. Values in the spring were definitely lower. Cameron [1928] further investigated the matter and found that the younger the rat the more variable was the serum-calcium and the more liable to be low in concentration. The curve for blood-calcium values appeared to be parallel with the curve for solar ultra-violet radiation, and Cameron suggests that the low levels frequently found in his young rats were the result of insufficient and seasonal variations in the vitamin D content of the foodstuffs fed. Cameron's figures were obtained from the study of a large number of rats and their accuracy is not to be doubted; nevertheless they cannot be taken as representing the normal picture for the rat living in this country under standard conditions, where the vitamin intake is unaffected by seasonal changes.

Magnesium. The serum-magnesium of the rat is approximately double that of man. The average value of the females did not change with age and was the same as for adult males. The young males, however, had definitely higher values. The difference between the average values of young and adult males was 0.93 mg. per 100 cc. The value of t is 5.856, whence P is less than 0.01 and the difference definitely significant. Had all the animals been examined irrespectively of age, there would still have appeared to be a difference between the males and females of 0.51 mg. per 100 cc., and this again is statistically significant ($t=4.907$ and P is less than 0.01). Charles [1931] found slightly lower serum-magnesium values in female rabbits compared with males, but the difference was not statistically significant for the number of animals used.

The lower serum-magnesium level of the young female rats is interesting in connection with magnesium deficiency. It has been the experience of the writer that females receiving a magnesium-deficient diet show pathological symptoms much more rapidly than do the males of the same age.

Correlation of serum-calcium and magnesium. Brookfield [1933] stated that there is an inverse relationship between the serum-calcium and magnesium of rabbits. No such inverse relationship was apparent from an inspection of the

figures given above for rats. An examination of the average values, on the contrary, indicates a tendency for these two serum constituents to vary in the same direction. The correlation coefficient of the 97 pairs of figures available for treatment is +0.36 and *P* is less than 0.01. There thus appears to be a definite, though not very rigid, relation between the serum-calcium and magnesium, but the coefficient being positive the ratio of the two is direct and not inverse.

SUMMARY.

1. The serum-calcium and magnesium values of approximately 100 rats have been determined.
2. Young rats had a slightly, but probably significantly, higher serum-calcium level than adult animals.
3. The females had a slightly lower serum-calcium than the males. The difference was not definitely significant.
4. The average serum-magnesium of adult males and of young and adult females was the same (4.4 mg. per 100 cc.), but young males had a significantly higher average (5.36 mg. per 100 cc.).
5. There was no seasonal variation in either mineral with the diet used.

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