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# METABOLISM OF $^{45}$Ca IN COCKERELS

*By*

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Thirty six Rhode Island Red cockerels, four months old were used to study the effect of different dietary calcium levels upon stable and radio-active calcium. They were divided equally into three groups fed on three rations containing 1.68, 1.97 and 0.02 calcium percent for normal, supplemented and deficient rations respectively. After two months, ten cockerels of each group were injected intramuscularly with $^{45}$Ca as $^{45}$Ca $cl_2$. One cockerel from each group was slaughtered at different intervals. Blood samples and some selected tissues were taken for chemical and radio active calcium assay.

The data obtained showed that the time required to renew the total amount of plasma calcium for deficent, normal and supplemented cockerels was 27.84, 10.84 and 16.83 hours respectively. $^{45}$Ca uptake percent by red muscles was higher than that uptake by white muscles in the three groups. The levels of $^{45}$Ca uptake percent by the liver fluctuated less than other soft tissues.

Slight differences of calcium content in soft tissues were found between the normal and supplemented groups, while those differences were observed among both these two groups and the deficient one. Calcium content in the tibia-bone shaft was higher than that of the tibia-bone upper end in the three groups. The results showed that the tibia-bone shaft is the main part controlling the calcium storage in the cockerels' body.

Calcium is present in body cells and is in some way necessary for their functioning. Approximately 99% of the calcium of the body is present in the bones and this percentage varied according to age, state of nutrition and species. The principal need for calcium during growth is for skeleton formation. Calcium is concerned for the coagulation of blood. It is also essential to the irritability of muscle, nerve and the rhythmic action of the heart. Calcium deficiency can upset the normal reporoductive performance.

During acute depletion of calcium with laying hens fed on a semipurified diet, the bone composition changes. The content of mg, Na, K and P in bone increase while the content of Ca decreases. (Taylor and Moore, 1956). Bregg *et al.* (1962), found that the biological half-life of $^{45}$Ca was 36 days for chicks fed on a ration containing 1.2% calcium. This biological

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half-life was increased by reducing the dietary calcium level, while decreased by increasing the dietary one. They found also that the dietary source of calcium as tested by calcium carbonate, calcium lactate and calcium sulphate appeared to have a different availability or absorption rate to the chicks but not sufficient to produce a significant change in the biological half-life.

## II—Experimental and Methods

1. *Birds :*

Thirty six Rhode-Island Red cockerels four months old with en average weight of about 815 gm. were used. They were divided into three equal groups and were placed individually in metabolic cages specially equiped to avoid contamination of cockerels with excreta. One group received the normal ration, while the two other groups received the supplemented and deficient ration respectively.

The composition of cockerels' rations was as shown in the following table :—

| Ingredients | Normal group | Supplemented group | Deficient group |
|---|---|---|---|
| Yellow corn % . . . . . | — | — | 100 |
| B-rley % . . . . . | 30 | 28.57 | — |
| Decorticated cottonseed meal % | 30 | 28.57 | — |
| Rice bran % . . . . . | 40 | 38.09 | — |
| Calcium carbonate % . . . . . | — | 4.77 | — |
| Ca % in the ration . . . . . | 1.682 | 1.974 | 0.018 |

2. *Application of radioisotope :*

After the pre-experimental period of two months on the three different rations, ten cockrels of each group were injecetd intramuscularly with $^{45}Ca$ as $^{45}Ca$. Each cockerel received 15 mc. radioactive calcium as a single dose

One cockerel of each group was slaughtered at 6, 12, 24, 48, 96, 144, 192, 244, 288 and 336 hours after injection. Ten ml. of blood, about 3 gm. of rectum contents and of some selected tissues were taken for chemical and radioactive calcium assay.

The radioactive calcium assay was carried out as recommended by Thomas *et al.* (1952).

3. *Calcium determination :*

To get rid of the phosphate ions, calcium solution of biological material samples was managed as the A.O.A.C. (1955). Calcium determination in the ash solution of biological materials, urine and in plasma using EDTA and ammonium purpurate indicator was carried out as described by Green blatt and Hartman (1951).

4. *Calculation :*

$$\text{The calcium uptake percent} = \frac{\text{counts/minute/gm. sample}}{\text{counts/minute/gm. live weight.}}$$

The disappearance rate of radioactive calcium (in the organ), the half-time value, and the turnover time were calculated as described by Comar (1955).

## III—Results and Discussion

*Total calcium and $^{45}Ca$ of cockerels' plasma*

The average values of cockerels' plasma calcium at the time of dosing were 12.50, 12.25 and 12.25mg/100ml for dficient, normal and supplemented cockerels respectively. Figure (1) showed that $^{45}Ca$ uptake percent decreased at a rapid rate till 96 hours after dosing. After 96 hours, the uptake percent by cockerels plasma increased at a very slow rate. This slight increase may be due to $^{45}Ca$ of bone resorption. Data from 6—96 hours after dosing were analyzed as recommended by Comar (1955). It was found that the time required to renew the total amount of calcium in the plasma (tt) of deficieent, noraml and supplemented cockerels was 27.84, 10.84, and 16.83 hours respectively. These results showed that decreasing or increasing the dietary calcium level, decreased the turnover rate of cockerel's plasma calcium.

*Tissue destribution of $^{45}Ca$ :*

Table (1) represents the $^{45}Ca$ uptake percent by white and red muscles of cockerels at successive hours. The results showed that decreasing or increasng the dietary calcium level decreased the time required for approaching th highest uptake percent of $^{45}Ca$ by white and red muscles. Generally, the $^{45}Ca$ uptake percent by red muscles was higher than that of white muscles. This means that calcium may be more important for red muscles than for white muscles of cockerles.

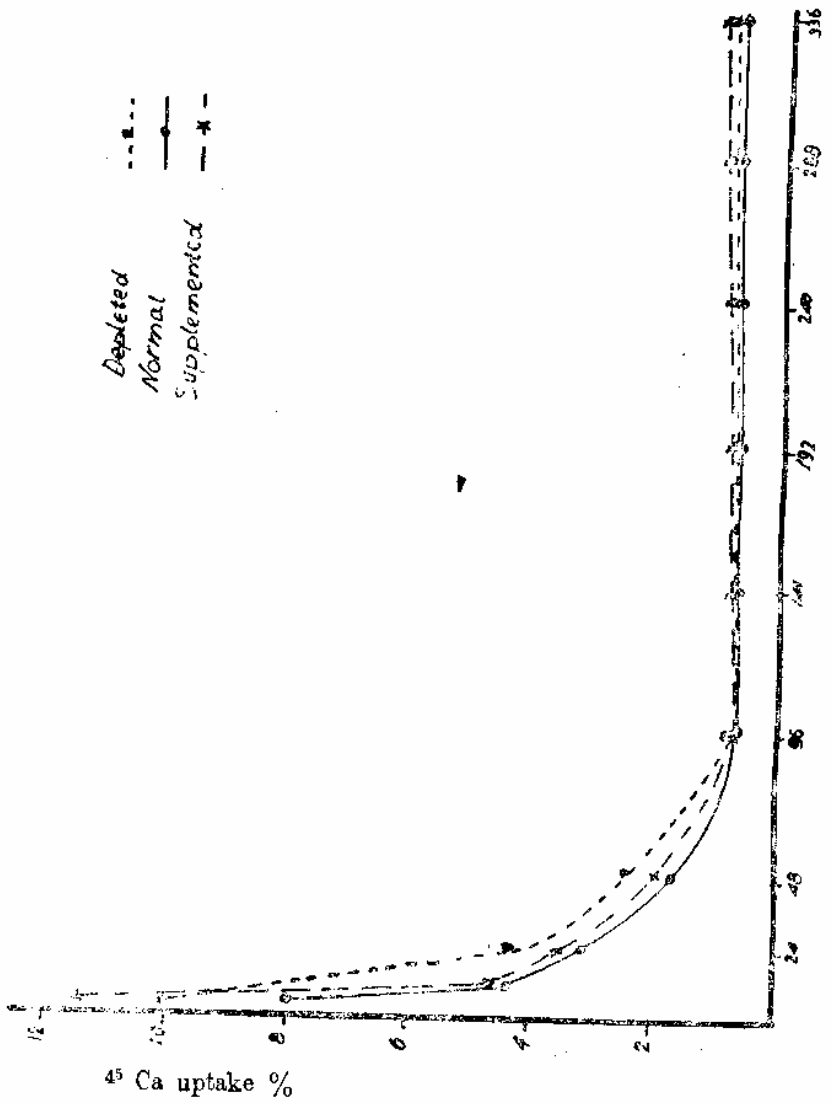


FIG. 1—$^{45}$Ca uptake % by Cockerels plasma.

TABLE 1.— THE EFFECT OF DIFFERENT DIETARY CALCIUM LEVELS UPON THE UPTAKE PERCENT OF RADIOACTIVE CALCIUM, $^{45}Ca$ BY WHITE AND RED MUSCLES OF COCKERELS.

| Hours after injection | White muscle | | | Red muscle | | |
|---|---|---|---|---|---|---|
| | Deficient | Normal | Supplemented | Deficient | Normal | Supplemented |
| | % | % | % | % | % | % |
| 6 | 2.88 | 4.30 | 0.32 | 6.74 | 3.10 | 9.52 |
| 12 | 7.93 | 1.74 | 3.55 | 11.65 | 1.98 | 3.66 |
| 24 | 2.55 | 7.47 | 3.06 | 3.97 | 12.60 | 5.08 |
| 48 | 2.22 | 1.11 | 2.18 | 1.15 | 6.47 | 1.17 |
| 96 | 0.26 | 0.20 | 4.73 | 0.73 | 0.67 | 2.00 |
| 144 | 1.73 | 4.94 | 3.26 | 11.50 | 6.95 | 2.94 |
| 192 | 1.07 | 0.40 | 1.24 | 1.00 | 4.91 | 0.95 |
| 244 | 1.25 | 1.66 | 3.00 | 1.71 | 2.47 | 2.08 |
| 288 | 1.46 | 2.20 | 1.50 | 0.66 | 1.49 | 1.00 |
| 336 | 1.55 | 3.20 | 0.76 | 0.00 | 0.00 | 0.00 |

Table (2) represents the $^{45}Ca$ uptake percent by cockerel's liver and kidneys at different successive hocrs. The level of $^{45}Ca$ uptake percent by the liver of the three groups decreased gradually from 144 hours after dosing. It reached zero at 288 hours for the deficient group. Generally the levels of $^{45}Ca$ uptake percent by liver fluctuated less than other soft tissues. This means that the liver may control calcium metabolism more than other soft tissues.

It can be noticed from Table (2) that the highest level of $^{54}Ca$ uptake percent by the kidney was at 6, 12 and 24 hours after dosing for supplemented, deficient and normal groups respectively. The level of $^{45}Ca$ uptake percent by the kidney of the three groups fluctuated at the different successive hours, and approached to be equal at 192 hours after dosing. It reached zero at 288 hours for the supplemented group. Hansard and Plumlee (1954), found

little differences between groups of rats in radioactive calcium percent retained by the various soft tissues. They pointed out, however, that a higher percentage of the $^{45}Ca$ dose was absorbed by those animals on the low-calcium diet.

TABLE 2.— THE EFFECT OF DIFFERENT DIETARY CALCIUM LEVELS UPON THE UPTAKE PERCENT OF RADIOACTIVE CALCIUM, $^{45}Ca$, BY LIVER AND KIDNEYS OF COCKERELS.

| Hours after injection | Liver | | | Kidney | | |
|---|---|---|---|---|---|---|
| | Deficient | Normal | Supplemented | Deficient | Normal | Supplemented |
| | % | % | % | % | % | % |
| 6 | 3.85 | 3.35 | 9.20 | 9.19 | 9.20 | 20.00 |
| 12 | 2.90 | 4.29 | 2.90 | 11.60 | 4.68 | 8.60 |
| 24 | 2.90 | 6.00 | 2.40 | 5.56 | 16.99 | 9.40 |
| 48 | 2.80 | 9.67 | 1.70 | 9.18 | 4.01 | 2.29 |
| 96 | 0.40 | 1.24 | 3.30 | 1.60 | 3.71 | 11.01 |
| 144 | 1.10 | 1.11 | 1.59 | 2.30 | 3.21 | 11.31 |
| 192 | 0.70 | 1.01 | 1.20 | 2.90 | 2.96 | 2.85 |
| 244 | 0.36 | 0.90 | 0.92 | 1.14 | 3.59 | 3.68 |
| 288 | 0.00 | 0.80 | 0.62 | 9.76 | 6.29 | 0.00 |
| 336 | 0.00 | 0.75 | 0.36 | 3.20 | 3.10 | 0.00 |

Table (3) represents the $^{45}Ca$ uptake percent by upper-end shoft of cockerels' tibia bone a different successive hours after injection. It can be noticed that the different parts of tibia bone contained more $^{45}Ca$ than soft tissues. This higher $^{45}Ca$ uptake percent was by the upperend and the lower one was by the shaft. This may be due to the fact tht the shaft is less exchangeable for the calcium than other tibia bone parts. This highest $^{45}Ca$ uptake percent by the upper-end was observed in deficient cockerels and the lowest values were in the supplemented ones. The highest $^{45}Ca$ that the lowest $^{45}Ca$ uptake percent by plasma and most selected soft tissues

was at 96 hours after injection. The values of $^{45}Ca$ uptake percent decreased gradually after the first 96 hours in the bone shaft, while they increased in the cockerels plasma. This may be due to the calcium resorption at shaft tibia-bone after that time.

Date presented in Table (3) were analyzed as recommended by Comar (1955). Results obtained from equations showed that the disappearance rate (K) of 45Ca per houre from the upper-end of tibia-bone was 0.115, 0.339 and 0.437%, and the time required to renew the half amount of calcium ($t^1/_2$) was 601.5, 204.2 and 158.6 hours for deficient, normal and supplemented cockerels respectively.

TABLE 3.— THE EFFECT OF DIFFERENT DIETARY CALCIUM LEVELS UPON THE UPTAKE PERCENT RADIOACTIVE CALCIUM, --Ca, BY TIBIA BONE OF COCKERELS.

| Hours after injection | Upper—end | | | Shaft | | |
|---|---|---|---|---|---|---|
| | Deficient | Normal | Supplemented | Deficient | Normal | Supplemented |
| | % | % | % | % | % | % |
| 6 | 250.12 | 245.13 | 252.11 | 50.21 | 71.52 | 51.41 |
| 12 | 220.15 | 182.11 | 149.19 | 76.35 | 86.43 | 76.21 |
| 24 | 380.23 | 369.31 | 288.10 | 89.47 | 100.80 | 87.51 |
| 48 | 288.01 | 196.03 | 105.10 | 84.50 | 85.51 | 85.42 |
| 96 | 320.04 | 257.14 | 160.03 | 185.08 | 122.60 | 12.73 |
| 144 | 280.11 | 204.23 | 137.14 | 146.01 | 101.71 | 101.69 |
| 192 | 230.22 | 144.12 | 120.43 | 111.24 | 80.84 | 80.07 |
| 244 | 210.04 | 120.02 | 98.41 | 70.51 | 60.50 | 64.14 |
| 288 | 200.01 | 100.18 | 80.08 | 64.60 | 50.71 | 54.06 |
| 336 | 178.40 | 80.15 | 60.20 | 60.08 | 36.60 | 36.71 |

The disappearance rate of $^{45}Ca$ from the shaft per hour was 0.103, 0.292 and 0.299% and ($t^1/_2$) was 66.7, 237.1 and 231.2 hours for deficient, normal and supplemented group respectively. These results are in good agrement with those obtained by Bregg *et al.* (1962). They found that increasing the dietary calcium level decreased $^{45}Ca$ level of the bone

They added that the disappearance of $^{45}Ca$ from the bone indicate that in addition to the formation of new bone salts in the growing cockerels, there was a constant turnover of deposited calcium. This was supported by the fact that the increase of the dietary calcium level decreased the biological half-life of $^{45}Ca$. The same authors suggested that this removal of calcium was probably a result of metabolic turnover and molecular exchange of stable dietary calcium with the radioactive calcium deposited in the bones.

It can be noticed from Table 1, 2 and 3 that the effect of different dietary calcium levels upon 45Ca uptake percent by bone was more obvious than that by soft tissues. From the disappearance rates of 45Ca, it can be deduced that the tibia-bone shaft is the main part controlling the calcium storage in the cockerels' body.

*Total calcium in different tissues.*

Calcium content (mg/100 gm) in selected fresh tissues of cockerels fed on different dietary calcium levels is presented in Table (4). It can be noticed that the calcium content in bones was higher than in soft tissues. Calcium content was the highest in the shaft being 465.0, 491.0 and 575.0 mg/100 gm for deficient, normal and supplemented cockerels.

TABLE 4.— THE EFFECT OF DIFFERENT DIETARY CALCIUM LEVELS UPON CALCIUM CONTENT (MG / 100 GM) IN SELECTED COCKERELS' TISSUES.

| Tissue | Deficient | Normal | Supplemented |
|---|---|---|---|
| White muscle | 3.606 | 3.922 | 3.940 |
| Red muscle | 4.004 | 4.302 | 4.568 |
| Kidney | 5.890 | 6.728 | 6.654 |
| Liver | 3.617 | 4.077 | 3.956 |
| Heart | 4.416 | 4.838 | 5.001 |
| Spleen | 2.096 | 2.773 | 2.575 |
| Tibia-bone upper end | 409.000 | 442.000 | 460.000 |
| Tibia-bone shaft | 465.000 | 491.000 | 575.000 |

Statistical analysis showed that the differences between calcium content in selected tissues of the three groups were insignificant as shown in the following table :

ANALYSIS OF VARIANCE

| Source of varionce | SS | MS | F |
|---|---|---|---|
| Between treatments . . . . | 2180.7 | 1090.2 | 0.02 |
| Within treatments . . . . | 1288286.3 | 53678.5 | |

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# « التمثيل الغذائى للكالسيوم فى الديوك النامية »

* ابراهيم الجندى ، محمد على رافت ، السيد رفعت ابو حسين
صبحى محمود علام

## الملخص

أجرى هذا البحث لمعرفة تأثير المستويات المختلفة من الكالسيوم فى الغذاء على توزيع الكالسيوم فى جسم الديوك النامية وذلك بالاستعانة بالكالسيوم المشع ( كا ٤٥ ) ولقد أجرى البحث على ستة وثلاثين ديكا من نوع الرود أيلاند الأحمر ــ قسمت الى ثلاثة مجاميع متساوية العدد والوزن.

غذيت هذه الديوك على علائق كانت نسبة الكالسيوم المئوية ٢ر٦٨٢ ، ١ر٩٧٤ ، ٠ر٢ فى العليقة الغنية والعادية والفقيرة فى الكالسيوم على التوالى . وبعد شهرين من بدء البحث أخذ عشر ديوك من كل مجموعة وحقنت بمقدار ١٥ ميكروكورى كالسيوم مشع على صورة كلوريد كالسيوم مشع عن طريق الحقن فى العضل لكل ديك . ثم ذبح ديك من كل مجموعة على فترات منتظمة واخذت عينات من الدم وبعض أجزاء الجسم وذلك لتقدير الكالسيوم كيماويا واشعاعيا . ولقد أمكن الحصول على النتائج الآتية :

١ ــ كانت النسبة المئوية للكالسيوم المشع فى البلازما متساوية فى المجاميع الثلاثة بعد ٩٦ ساعة بعد الحقن . ووجد أن الوقت اللازم لتجديد كل الكالسيوم الموجود فى البلازما هى ٢٧ر٨٤ ، ١٠ر٨٤ ، ١٦ر٨٣ ساعة فى الديوك المغذاة على عليقة فقيرة وعادية وغنية بالكالسيوم على التوالى .

٢ ــ كانت النسبة المئوية للكالسيوم المشع فى العضلات الحمراء اكثر منها فى العضلات البيضاء وكان مستوى الكالسيوم المشع فى الكبد أقل تغيرا من بقية أجزاء الجسم الأخرى الطرية .

٣ ــ لوحظ أن الجزء الأوسط من عظمة الساق اكثر الأجزاء تحكما فى تخزين الكالسيوم الموجود فى جسم الديوك .

٤ ــ اظهرت التحليلات الاحصائية أن الفروق فى محتويات أجزاء الجسم من الكالسيوم فى حالة المجاميع المختلفة غير معنوية .

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