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# EFFECT OF DEXAMETHASONE ON CALCIUM METABOLISM AND $^{47}Ca$ KINETICS IN NORMAL SUBJECTS

By

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## ABSTRACT

The effects of pharmacological doses of dexamethasone on calcium metabolism were evaluated in 4 normal subjects, by stable calcium and phosphorus balances and $^{47}Ca$ kinetic studies. The radioactivity data were satisfactorily fitted to a model with 2 exchanging compartments.

There was a significant increase in urinary calcium excretion rate with higher specific activities. The total faecal calcium did not alter despite changes in its components, i. e. a fall in endogenous faecal calcium and an increase in unabsorbed dietary calcium. The data suggest that dexamethasone inhibits the rate of calcium transfer across the intestinal wall, more intensely from the mucosal cell to the lumen (secretion).

From the constants of compartmental analysis, the only significant and consistent change was the increase in bone resorption rate. Bone deposition rate increased in 2 and decreased in the remaining 2 subjects.

The results of our studies indicate that dexamethasone has a direct effect on the way calcium is dealt with by the kidney and the gut and that the drug has a direct effect on the skeleton.

For comparison a patient with Cushing's syndrome was studied during the active phase and also after clinical and laboratorial remission of the disease.

Treatment with glucocorticoids causes negative calcium balance and loss of bone mass in human adults – osteoporosis, which is a common and serious complication, since it is found in 40 to 83 % of patients in several large series of natural chronic hypercortisonism (*Riggs et al.* 1966).

However, this aspect of calcium metabolism, probably reflecting an imbalance between bone deposition and resorption rates of calcium (*Eisenberg* 1964), is not the only manifestation of high blood levels of cortisol or of one of its synthetic analogues. It has also been shown that glucocorticoids modify calcium transport in the renal tubule (*Laake* 1960) and inhibit intestinal absorption of calcium in rats (*Stoerk & Arison* 1961) and dogs (*Collins et al.* 1962), particularly in the latter where the major route of $^{47}Ca$ excretion is via the faeces. However, in man, the effect of glucocorticoids on calcium absorption has been less studied (*Bentzel et al.* 1964).

The overall effect of glucocorticoids on calcium metabolism is still a matter of controversy and the information obtained in man is almost exclusively derived from the study of the effects of cortisol or its synthetic analogues in patients with chronic diseases. The results obtained are rather difficult to interpret because of the special characteristic of the primary disease and its possible interference with the action of corticoid on calcium metabolism.

Apart from the work of *Eisenberg* (1966) there are no systematic studies of the effects of pharmacological doses of corticoids on calcium metabolism in normal volunteers using radioactive calcium or other tracer of this element. However, there are two reservations about the kinetic analyses performed by *Eisenberg* (1966): first he used non-radioactive strontium as a tracer for calcium and it is known that strontium is dealt with somewhat differently from calcium in the body (*Bauer* 1964). Secondly the kinetic model was based on a single exchange compartment, which is an over-simplification and thus unacceptable for many reasons (cit. in *Heaney* 1964).

This report describes studies of calcium kinetics in a group of four young healthy adult males studied before and after dexamethasone treatment. Combined metabolic balance and isotopic tracer techniques were used.

For comparison a patient (A. J. O., 30 years, male) with long-standing Cushing's syndrome (2 years) was studied before and after remission of the disease.

## MATERIAL AND METHODS

The normal subjects were four healthy men aged 17 to 28. They were studied before and after dexamethasone (9$\alpha$-fluoro-11$\beta$,17,21-trihydroxy-16$\alpha$-methyl-pregnan-1,4-diene-3,20-dione) treatment (9 mg/day given orally in 4 divided doses) for 27 days. Two 9-day balance studies were done, before and after 18 days of dexamethasone treatment.

Experimental design: Each of the subjects was kept on a constant calcium and phosphorus diet (708 to 1243 mg calcium and 1008 to 1420 mg phosphorus daily – see Table 1), containing 150 meq. of sodium and 100 meq. of potassium. At least 3 complete analyses of dietary homogenates were made for each 9-day study to determine the intake. The same diet was maintained during the period of dexamethasone treatment. One week of adjustment was allowed before the start of the experiment.

Analyses of calcium and phosphorus were made from 3-day urine and stool collections (3) during the 9-day control and dexamethasone study.

Stools, diets, plasma and urine were analyzed for calcium and phosphorus by the methods of Kramer and Tisdall modified by *Clark & Collip* (1923) and *Fiske & Subbarow* (1925), respectively.

*Calcium kinetics:* 40 $\mu$c of $^{47}Ca\ Cl_2$ dissolved in 10 ml of sterile isotonic saline were given intravenously on the morning of the first day of a balance period. Blood samples were drawn at 10 min, 6 h and 12 h on the day of injection and then twice daily, at 8 a. m. and 8 p. m., for the remaining 8 days. Aliquots of 3-day pools of urine and stool homogenates were counted. Five-ml samples of plasma and urine and a weighed sample of stools, of a similar volume, were counted in a well scintillation counter ($5 \times 5$ cm NaI (Tl) crystal) with a pulse height analyser set to eliminate the contribution from $^{47}Ca$. Sufficient counts were collected to an accuracy of 1.2 % with a probability of 95 %.

The total dosage per patient for the two studies did not exceed the 89 $\mu$c suggested as the safe upper limit in any 13-week period by *Corey et al.* (1961).

The regression analysis of the plasma specific activity curve (as illustrated in Fig. 1), limited to the first five to seven days*, can be satisfactorily fitted to the sum of two exponential terms. Hence, two exchanging compartments were accepted as the kinetic model (Fig. 2).

The symbols, definitions and units used in the present analysis are as follows:

| *Symbols* | *Definitions* | *Units* |
|---|---|---|
| $S_n$ | Size of compartment *n* | Mass (grams) |
| $R_n$ | Amount of tracer in compartment *n* | Counts per minute (Cpm)<br>% injected dose |
| $X_n$ | Specific activity of tracer in compartment *n* ($X_n = R_n / S_n$) | Cpm/mass or % injected |
| $X_{no}$ | Specific activity of tracer in compartment *n* at time zero | Cpm/mass or % injected dose/mass |
| $Ro_{nr}$ | Rate of exchange (mass) between compartments *n* and *r* | Mass/time |
| $K_{nr}$ | Fraction of S transferred to compartment *r* in unit time ($K_{nr} = Ro_{nr} / S_n$) | Fraction/time |

* The analysis was limited to the 5–7 days period because at this time ($\Theta$), a break occurs in the slope of the curves for all subjects. At this time there is a slight rise in plasma specific activity and/or an upward displacement of the slope suggestive of a sudden enrichment of the exchangeable pool from a source of calcium with higher specific activity than the pool. This phenomenon may result from the resorption of bone with high specific activity, which has been laid down shortly after the injection of $^{47}Ca$, acting as an endogenous source of isotope for the exchangeable pool (*Lafferty & Pearson* 1964).

In this model, compartment *1* represents all the calcium distribution spaces which mixes internally in less than 15 min. This »rapidly miscible pool« includes the plasma in which the tracer is introduced. Compartment *2* represents the physiological pool of calcium in isotopic equilibrium with compartment *1* within 2–4 days, comprising part of the exchangeable bone, that of relative slow exchange (*Rich et al.* 1961; *Heaney* 1964).

Other models with a greater number of compartments (4) have been suggested (*Bauer & Ray* 1958; *Aubert & Milhaud* 1960; *Garrett et al.* 1962; *Neer et al.* 1967). If blood sampling was prolonged over 18 to 20 days, four exponentials were also sufficient to satisfy the data in *Neer et al.*'s (1967) kinetic studies. However, the majority of proposed models can be simplified to a two-compartment model similar to that used in our study; three of the proposed four compartments equilibrate with each other within minutes, and in the fourth, only after 2–3 days (*Heaney* 1964).

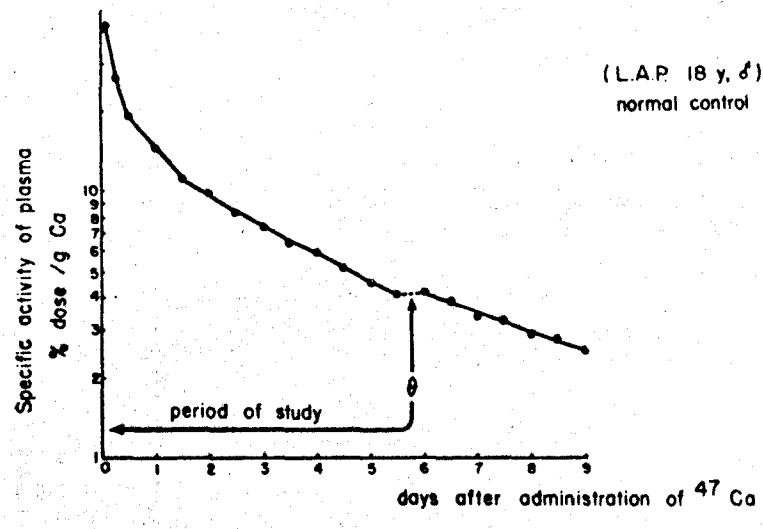


*Fig. 1.*
Semi-logarithm plot of $^{47}$Calcium plasma specific activities as a function of time.

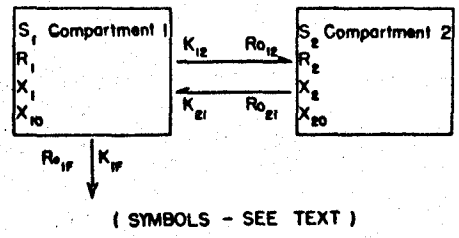


*Fig. 2.*
Compartmental model of calcium kinetics.

In the model (Fig. 2) calcium is irreversibly lost from compartment *1* at a rate of $Ro_{14}$ to urine and to faeces at a rate of $Ro_{15}$. Urinary calcium is lost from compartment *1* because the cumulative urinary radioactivity is directly proportional to the integral of the plasma specific activity. Faecal calcium is assumed to come directly from compartment *1* since it seems anatomically reasonable and the data cannot explain calcium mixing in the gut. For the same reasons calcium absorbed from the diet is assumed to enter compartment *1* directly. Urinary and faecal radioactivity do not account for all the $^{47}Ca$ lost from compartment *1*, at a rate of $Ro_{1F}$. The remainder represents internal loss to »nonexchangeable or stable bone« at a rate of $Ro_{13}$. Therefore, $Ro_{1F}$ corresponds to the total amount of calcium transferred out of compartment *1* per unit of time ($Ro_{1F} = Ro_{14} + Ro_{15} + Ro_{13}$); an assumption that is valid if no excretion or deposition occurs from pool 2. It has been pointed out that part of the calcium transferred from the exchangeable pool (compartment *1*) to bone may be accounted for by new formation, and part by exchange between some major fraction of skeletal calcium and the relatively small miscible pool. The importance of the second mechanism is that it affects the exponential disappearance of tracer from the rapidly exchangeable calcium pool which is unaccompanied by a net movement of stable calcium (*Heaney & Whedon* 1958). For this reason, like *Eisenberg & Gordan* (1961), we have called the entire movement of tracer from the exchangeable pool to deeper layers of bone (stable bone), the bone deposition rate.

From the accepted model, the specific activity of the tracer in compartment *1* is described by the following differential equation:

$$X_1 = C_1 e^{\lambda_1 t} + C_2 e^{\lambda_2 t}$$

where $C_1$ and $C_2$ are the coefficients and $\lambda_1$ and $\lambda_2$ the fractional loss rate constants for compartments *1* and *2* respectively.

The plasma data were fitted by means of an iterative process using a programme for the IBM-1620 computer similar to that described by *Berman et al.* (1962). From the fitting of the kinetic data, the coefficients $C_1$, $C_2$, $\lambda_1$ and $\lambda_2$ were obtained and thus values could be derived for the parameters of the model, i. e., compartmental sizes ($S_1$ and $S_2$) and flow rates ($K_{21}$, $K_{12}$ and $K_{1F}$).

As the calculated urinary calcium is in agreement within 5 % with the chemically determined urine calcium (*Lafferty & Pearson* 1963) $Ro_{14}$ can be expressed as the average daily urinary stable calcium (mg/day) and is calculated from 0 to 6 days to coincide more closely with the period used for our compartmental studies.

As far as $Ro_{15}$ – faecal excretion of calcium of endogenous origin ($Ca_Fe$) – is concerned, this corresponds to the fraction of the calcium entering the gut with the digestive juices (Ca d. j.) which fail to be reabsorbed and thus appear in the faeces.

Knowing the quantity of tracer cleared per unit of time and the average concentration of tracer per unit substance cleared, we can obtain the quantity of substance cleared per unit of time, based on the usual formula for the measurement of clearances. Thus $Ro_{15}$ can be defined (*Heaney & Skillman* 1964) as follows:

$$Ro_{15} = \text{faecal } ^{47}Ca \left\{ \begin{matrix} t_2 \\ t_1 \end{matrix} \Big/ \int_{t_1}^{t_2} X_1 \cdot dt \right.$$

where $Ro_{15}$ is given as grams Ca per day; $X_1$ is the plasma calcium specific activity; $t_1$ and $t_2$ are two time intervals during the study. The numerator of the equation is the total radioactivity in the pooled faeces over the relevant time interval. In our study $t_1$ was set at zero, i. e., the time of isotope injection, and $t_2$ at the completion of the study, thus providing a collection period of 9 days.

Having obtained the values for $Ro_{14}$ and $Ro_{15}$, $Ro_{13}$ (bone deposition rate) can be readily determined, by the difference, also expressed in grams/day:

$$Ro_{13} = Ro_{1F} - (Ro_{14} + Ro_{15})$$

Since changes in calcium balance largely reflect the balance between bone formation and bone resorption (*Lafferty & Pearson* 1963), total skeletal resorption (R) may be calculated as follows:

$$R = Ro_{13} - \text{Calcium balance per day.}$$

This assumption is valid only if no calcium is deposited in tissues other than bone.

As far as intestinal calcium absorption, unabsorbed ($Ca_D$ n. a.) and absorbed ($Ca_D$ a) dietary calcium were determined in the following manner as suggested by *Blau et al.* (1959)

$$Ca_D \text{ n. a.} = Ca_F - Ca_Fe$$

where $Ca_F$ is the average daily faecal calcium and $Ca_Fe$, as mentioned above, the endogenous faecal calcium ($Ro_{15}$)

$$Ca_D \text{ a} = Ca_D - Ca_D \text{ n. a.}$$

where $Ca_D$ is the daily calcium intake.

For comparative studies, *Heaney & Skillman* (1964) have defined the fractional calcium absorption, designated by the symbol $\alpha$:

$$\alpha = Ca_D \text{ a} / Ca_D$$

Its determination requires no moldels or assumptions and depends only on the ability to measure $Ca_Fe$, $Ca_F$ and $Ca_D$.

If we accept that the calcium of digestive juices (Ca d. j.) is dealt with by the gut with approximately the same efficiency as calcium of dietary origin and that its absorbable and unabsorbable fractions are the same for hyper- and hypo-absorbers, then we can calculate the Ca d. j. since the endogenous faecal excretion of calcium and the fractional dietary calcium absorption are known (*Heaney & Skillman* 1964):

$$Ca_Fe = \text{Ca d. j.} - (\alpha \cdot \text{Ca d. j.})$$

then

$$\text{Ca d. j.} = Ca_Fe / 1 - \alpha$$

It is more reasonably to suggest that Ca d. j. is subject to a continuum of absorption efficiencies, related in part to the site of entrance of calcium into the digestive tract, but it is not easy to resolve a mathematical model which incorporates continuously absorption efficiencies. Thus, the real value for Ca d. j. must lie within a range limited at the bottom by the $Ca_Fe$ and at the top by a $Ca_Fe / 1 - \alpha$, i. e.,

$$Ca_Fe \leq Ca \text{ d. j.} \leq Ca_Fe / 1 - \alpha$$

The calculated value, in which the $Ca_Fe$ is corrected for absorption, producing the maximum possible value for Ca d. j., was the one used in our study for estimating Ca d. j. This is what is generally done by other investigators (*Blau et al.* 1954, 1959).

Paired comparisons, according to *Goulden* (1952), were done to evaluate the significance of the results obtained in the normal subjects before and after dexamethasone treatment. The ratios control / dexamethasone were submitted to the transformation Arcosin $\sqrt{\text{ratio}}$ according to *Snedecor* (1956) in order to satisfy more nearly the assumption that the populations are normally distributed. The level of 5 % was accepted as significant.

## RESULTS

### 1. *Stable calcium and phosphorus balances*

Table 1 summarizes the balance data before and after dexamethasone treatment in the 4 normal subjects and the patient with Cushing's syndrome during the active phase of the disease and 11-months after pituitary irradiation and 7 months after left adrenalectomy and in complete clinical remission. The mean plasma values for calcium and phosphorus are also indicated.

During the control phase of study, all normal subjects were in positive calcium and phosphorus balance. During dexamethasone treatment, all patients demonstrated an increased rate of urinary calcium excretion, resulting in a negative balance in 3 of the subjects and a reduction in the positive balance in the remaining subject L. A. P. from +111 to +16 mg/24 h. There were no significant changes in faecal calcium. Phosphorus balance showed analogous but more intense changes after the synthetic steroid.

Mean plasma calcium levels did not change significantly after dexamethasone (before $\bar{x} = 9.95$ mg/100 ml; after $\bar{x} = 10.06$ mg/100 ml; $t = 0.141$; $t_{0.05} = 2.353$).

Similarly, the mean plasma inorganic phosphorus did not reveal any change after dexamethasone treatment (before $\bar{x} = 3.40$ mg/100 ml; after $\bar{x} = 3.34$ mg/100 ml; $t = 0.659$).

In the patient with Cushing's syndrome, the calcium balance changed from a slightly to a strikingly positive value after clinical and laboratorial remission of the disease. This situation resulted almost entirely from the marked re-

*Table 1.*
Calcium and phosphorus balances.

| Patient | Age years | Weight (kg) | Dexa-meth. (days) | Period | Calcium (mg/24 h) | | | | Phosphorus (mg/24 h) | | | | Plasma | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Intake | Urine | Faeces | Balance | Intake | Urine | Faeces | Balance | Ca* mg/100 ml | P* mg/100 ml |
| E. P. | 17 | 67.3 | — | 9 days | 1026 | 57 | 913 | + 56 | 1130 | 504 | 588 | + 38 | 10.35 | 3.59 |
| | | 64.0 | 27 | 9 days | 1026 | 122 | 924 | — 20 | 1234 | 1030 | 634 | —430 | 10.48 | 3.40 |
| L. A. P. | 18 | 64.8 | — | 9 days | 1227 | 380 | 736 | +111 | 1327 | 452 | 562 | +313 | 9.69 | 3.12 |
| | | 60.5 | 27 | 9 days | 1243 | 526 | 701 | + 16 | 1042 | 462 | 552 | + 28 | 9.96 | 3.26 |
| W. M. | 22 | 58.0 | — | 9 days | 735 | 213 | 402 | +120 | 1048 | 473 | 356 | +219 | 9.93 | 3.64 |
| | | 55.5 | 27 | 9 days | 708 | 352 | 547 | —191 | 1041 | 1300 | 420 | —679 | 9.83 | 3.40 |
| J. A. R. | 23 | 60.9 | — | 9 days | 1224 | 325 | 766 | +133 | 1248 | 486 | 319 | +443 | 9.82 | 3.24 |
| | | 54.7 | 27 | 9 days | 1210 | 617 | 669 | — 76 | 1008 | 1034 | 351 | —377 | 10.48 | 3.28 |
| A. J. O.** | 30 | 73.6 | pre-op. | 9 days | 1003 | 41 | 915 | + 47 | 1088 | 509 | 346 | +565 | 10.48 | 2.46 |
| | | | remission | 9 days | 1036 | 18 | 585 | +633 | 1420 | | | | 9.43 | 3.52 |

* Mean values for each period.
** Cushing's syndrome (bilateral adrenal hyperplasia).

*Table 2.*
$^{47}Ca$ balance data.

| Patient | Treatment | Faeces* $^{47}Ca$ (% injected dose) | | | Urine* $^{47}Ca$ (% injected dose) | | | »Body« retention** $^{47}Ca$ (% injected dose) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 days | 6 days | 9 days | 3 days | 6 days | 9 days | 3 days | 6 days | 9 days |
| E. P. | Control | 5.16 | 8.41 | 10.68 | 1.20 | 1.84 | 2.55 | 93.64 | 87.75 | 86.79 |
| | Dexamethasone | 5.50 | 9.82 | 13.25 | 5.04 | 6.75 | 7.82 | 89.46 | 83.25 | 78.93 |
| W. M. | Control | 3.25 | 7.42 | 8.44 | 8.08 | 11.27 | 14.20 | 88.67 | 81.31 | 77.36 |
| | Dexamethasone | 0.12 | 0.74 | 4.43 | 16.83 | 24.26 | 30.20 | 83.05 | 74.43 | 65.37 |
| J. A. R. | Control | 4.36 | 8.56 | 10.65 | 5.65 | 8.22 | 9.52 | 89.99 | 83.22 | 79.83 |
| | Dexamethasone | 0.16 | 4.45 | 7.81 | 14.31 | 21.55 | 25.18 | 85.53 | 74.00 | 67.01 |
| L. A. P. | Control | 4.52 | 6.20 | 7.39 | 3.72 | 5.35 | 6.33 | 92.03 | 88.45 | 86.28 |
| | Dexamethasone | 4.40 | 6.54 | 8.38 | 12.00 | 16.64 | 19.38 | 83.60 | 76.82 | 72.24 |
| A. J. O. | Remission | 4.60 | 7.27 | 8.52 | 0.57 | 0.76 | 0.89 | 94.83 | 91.97 | 90.59 |
| | Cushing's | 5.65 | 12.05 | 17.53 | 1.32 | 2.19 | 3.09 | 93.03 | 85.76 | 79.38 |

* Cumulative excretion.
** Cumulative retention.

duction in faecal calcium, with a slight decrease in urinary calcium, already low in the active phase of the disease.

The mean plasma calcium level was kept within the confidence interval for single normal values (9.33 mg/100 ml – 10.77 mg/100 ml). On the other hand, during the active disease there was a significant hypophosphataemia in relation to the confidence interval for normal values for plasma inorganic phosphorus (2.93 mg/100 ml – 3.87 mg/100 ml).

2. *$^{47}Ca$ balances*

Renal and faecal $^{47}Ca$ excretion as percentage of the injected dose and body $^{47}Ca$ retention (% dose injected – % dose excreted in the urine and faeces), before and after dexamethasone treatment and in the patient with Cushing's syndrome are indicated in Table 2. Comparison of the cumulative excretion (9 days) of the isotope in the urine and faeces and its body retention, in the same time interval, before and after the induction of hypercortisonism, indicated that there was a significant increase in the Arcosin $\sqrt{\%}$ dose excreted in the urine ($t = 9.021$) and a significant reduction in the Arcosin $\sqrt{\%}$ body retention ($t = 9.544$) and no significant change at the 5 % level ($t = 2.353$) in the Arcosin $\sqrt{\%}$ faecal excretion between the period before and after dexamethasone ($t = 0.108$).

The patient with Cushing's syndrome, in remission, behaved like the normal subjects and in the active phase like the same control subjects during the induction of acute hypercortisonism.

3. *Compartmental analysis*

The solution for the values of the parameters of the model accepted in our study (Fig. 2) – constants of compartmental analysis – for both periods of study in the normal subjects and in the patient with Cushing's syndrome are presented in Table 3.

The mean values for the constants of compartmental analysis are shown in Table 4.

Dexamethasone treatment, induced, in the 4 normal subjects, as the only statistically significant changes, an increase in urinary calcium levels ($Ro_{14}$) ($t = 3.120$), a fall in endogenous faecal calcium ($Ro_{15}$) ($t = 4.314$) and an increase in bone resorption rate (R) ($t = 2.930$). No significant changes more demonstrated in the remaining parameters for $t_{0.05} = 2.353$.

In the patient A. J. O., in remission from Cushing's syndrome, all constants of compartmental analysis were within the confidence interval for single nor-

*Table 3.*
Constants of compartmental analysis.

| Patient | Period | $Ro_{1F}$ (g/day) | $Ro_{14}$ (g/day) | $Ro_{15}$ (g/day) | $Ro_{13}$ (g/day) | $Ro_{12,21}$ (g/day) | Compartment 1 ($S_1$) (g) | Compartment 2 ($S_2$) (g) | Total exchangeable Ca (1 + 2) (g) | R (g/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| E. P. | Control | 1.516 | 0.052 | 0.238 | 1.226 | 4.409 | 1.519 | 3.505 | 4.824 | 1.141 |
| | Dexamethasone | 1.359 | 0.097 | 0.141 | 1.121 | 5.915 | 1.256 | 2.582 | 3.838 | 1.170 |
| L. A. P. | Control | 1.360 | 0.380 | 0.441 | 0.539 | 4.718 | 1.134 | 2.680 | 3.814 | 0.428 |
| | Dexamethasone | 1.335 | 0.522 | 0.204 | 0.609 | 5.193 | 1.267 | 2.363 | 3.630 | 0.593 |
| W. M. | Control | 1.269 | 0.216 | 0.143 | 0.910 | 3.901 | 1.371 | 2.497 | 3.868 | 0.790 |
| | Dexamethasone | 1.342 | 0.359 | 0.011 | 0.972 | 4.031 | 1.057 | 2.789 | 3.846 | 1.163 |
| J. A. R. | Control | 1.053 | 0.329 | 0.342 | 0.382 | 4.100 | 0.821 | 2.435 | 3.276 | 0.249 |
| | Dexamethasone | 1.219 | 0.621 | 0.241 | 0.357 | 4.286 | 1.007 | 2.417 | 3.424 | 0.432 |
| A. J. O. | Remission | 1.379 | 0.018 | 0.172 | 1.189 | 4.914 | 1.385 | 2.674 | 4.059 | 0.556 |
| | Cushing's | 0.961 | 0.041 | 0.225 | 0.715 | 5.272 | 2.347 | 1.529 | 3.876 | 0.668 |

$Ro_{1F} = Ro_{14} + Ro_{15} + Ro_{13}$ (Ca loss rate from compartment 1)
$Ro_{14}$ = Urinary Ca excretion rate
$Ro_{15}$ = Endogenous faecal Ca ($Ca_Fe$) excretion rate
$Ro_{13}$ = Ca transfer rate to bone
$Ro_{12}$ = Ca flow rate from compartment 1 to 2
$Ro_{21}$ = Ca flow rate from compartment 2 to 1
R = Ca transfer rate bone to Exchangeable pool

*Table 4.*
Average values for the constants of compartmental analysis.

| Study | $Ro_{1F}$ (g/day) | $Ro_{14}$ (g/day) | $Ro_{15}$ (g/day) | $Ro_{13}$ (g/day) | $Ro_{12,21}$ (g/day) | Compartment 1 (g) | Compartment 2 (g) | Total exchangeable (g) | Resorption (g/day) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1.299 | 0.244 | 0.291 | 0.764 | 4.282 | 1.211 | 2.734 | 3.945 | 0.652 |
| Dexamethasone | 1.314 | 0.400* | 0.149* | 0.765 | 4.856 | 1.147 | 2.538 | 3.684 | 0.839* |
| Remission | 1.379 | 0.018 | 0.172 | 1.181 | 4.914 | 1.385 | 2.674 | 4.059 | 0.536 |
| Cushing's | 981* | 0.041* | 0.225 | 0.715* | 5.272 | 2.347 | 1.529* | 3.876 | 0.668 |

* Statistically significant change ($P < 0.05$)

*Table 5.*
Constants of intestinal absorption of calcium.

| Patient | Period | $Ca_D$ (g/day) | $Ca_F$ (g/day) | $Ca_Fe$ (g/day) | $Ca_D$ n. a. (g/day) | $Ca_D$ a (g/day) | $\alpha$ | Ca d. j. (g/day) | $Ca_Fe$/Ca d. j. × 100 |
|---|---|---|---|---|---|---|---|---|---|
| E. P. | Control | 1.026 | 0.913 | 0.238 | 0.675 | 0.351 | 0.342 | 0.362 | 65.8 |
| | Dexamethasone | 1.026 | 0.924 | 0.206 | 0.718 | 0.308 | 0.300 | 0.294 | 70.1 |
| L. A. P. | Control | 1.227 | 0.736 | 0.443 | 0.293 | 0.394 | 0.842 | 2.804 | 15.8 |
| | Dexamethasone | 1.243 | 0.701 | 0.227 | 0.474 | 0.769 | 0.618 | 0.594 | 38.2 |
| W. M. | Control | 0.735 | 0.402 | 0.126 | 0.276 | 0.459 | 0.624 | 0.335 | 37.6 |
| | Dexamethasone | 0.708 | 0.547 | 0.051 | 0.496 | 0.212 | 0.299 | 0.073 | 69.9 |
| J. A. R. | Control | 1.244 | 0.766 | 0.363 | 0.403 | 0.821 | 0.670 | 1.100 | 33.0 |
| | Dexamethasone | 1.210 | 0.669 | 0.191 | 0.478 | 0.732 | 0.605 | 0.484 | 39.5 |
| A. J. O. | Remission | 1.036 | 0.385 | 0.172 | 0.213 | 0.823 | 0.794 | 0.835 | 20.6 |
| | Cushing's | 1.003 | 0.915 | 0.233 | 0.682 | 0.321 | 0.320 | 0.343 | 67.9 |

$Ca_D$ = Calcium intake
$Ca_F$ = Faecal calcium
$Ca_Fe$ = Endogenous faecal calcium
$Ca_D$ n. a. = Unabsorbed dietary calcium
$Ca_D$ a = Absorbed dietary calcium
$\alpha$ = Fractional calcium absorption ($Ca_D$ a / $Ca_D$)
Ca d. j. = Calcium of digestive juices ($Ca_Fe$ / 1 – $\alpha$)

mal values. At the active stage, when compared with the period of acute induced hypercortisonism in the normal controls, there was a significant fall in $Ro_{1F}$, $Ro_{13}$ and $S_2$ and an increase in $Ro_{14}$.

4. *Intestinal absorption of calcium*

Table 5 shows the values for the constants of intestinal absorption of calcium in the normal subjects, before and after dexamethasone treatment as well as the results in the patient with Cushing's syndrome. The ratio $Ca_Fe$ / Ca d. j. is also shown in Table 5.

As the dietary calcium varied from 0.736 to 1.227 g daily we were unable to establish the confidence interval for single values for the several constants of intestinal absorption of calcium in considering their dependence on the amount of ingested calcium (*Malm* 1958; *Heaney & Skillman* 1964; *Nordin & Smith* 1965). Therefore, the values for the constants of intestinal absorption were expressed by the ratios of the constants to the dietary calcium. These ratios allowed us to establish the confidence limits for the normal values.

The comparison of the constants of intestinal absorption (expressed as indicated in the normal subjects, before and after the induction of acute hypercortisonism) showed that there was a significant increase in unabsorbed dietary calcium (Ca n. a.) ($t = 3.107$) and in the ratio $Ca_Fe$ / Ca d. j. ($t = 2.503$), a significant decrease in endogenous faecal calcium ($Ca_Fe$ a) ($t = 3.135$), in absorbed dietary calcium ($Ca_I$ a) ($t = 3.041$), in fractional calcium absorption ($\alpha$) ($t = 2.515$) and of calcium of digestive juices (Ca d. j.) ($t = 4.413$).

The patient with Cushing's syndrome, in remission, had all the constants of intestinal absorption within the confidence interval for normal single values.

At the active stage of the disease, when compared with the acute dexamethasone-induced hypercortisonism in the normal subjects, patients A. J. O. showed a significant increase in faecal calcium, as mentioned above, and also an increased $Ca_Fe$ and $Ca_I$ n. a. in relation to the remission period.

5. *Urinary, faecal and systemic specific activities*

Table 6 shows the urinary, faecal and systemic specific activities, the latter being indicated by the ratio % $^{47}$Ca retained / Exchangeable pool (E), in the patients studied.

The comparison in the normal subjects, of the pre-period with the post-steroid period, indicated a significant increase in the ratio % $^{47}$Ca urine / $^{40}$Ca urine ($t = 7.575$), a decrease in % $^{47}$Ca faeces / $^{40}$Ca faeces ($t = 2.221$) for $t_{0.05} = 2.353$ corresponding to 3 degrees of freedom.

*Table 6.*
Urinary, faecal and systemic specific activities.

| Patient | Period | % Dose urine* / $Ca_U$ | % Dose faeces* / $Ca_F$ | % Dose retained* / E |
|---|---|---|---|---|
| E. P. | Control | 44.74 | 11.68 | 20.57 |
| | Dexamethasone | 64.10 | 14.34 | 17.99 |
| L. A. P. | Control | 16.66 | 10.04 | 22.62 |
| | Dexamethasone | 36.84 | 11.95 | 19.90 |
| W. M. | Control | 66.67 | 20.99 | 20.00 |
| | Dexamethasone | 85.80 | 7.92 | 16.99 |
| J. A. R. | Control | 29.29 | 13.90 | 24.36 |
| | Dexamethasone | 40.81 | 11.67 | 19.57 |
| A. J. O. | Remission | 49.44 | 22.13 | 22.32 |
| | Cushing's | 75.37 | 19.16 | 20.48 |

* % Dose accumulated up to the 9th day.

The patient A. J. O. when in remission, behaved like normal subjects in the control period, and like normal subjects after the induction of hypercortisonism, in the active period of the disease.

## DISCUSSION

1. *Effect on urinary calcium*

The present studies indicate a very significant increase in urinary excretion rate of calcium, as indicated by $^{40}Ca$ (Table 1) and $^{47}Ca$ (Table 2) balances. This marked hypercalciuria has been repeatedly demonstrated by the AA. in spontaneous and induced hypercortisonism (*Albright & Reifenstein* 1948; *Pechet et al.* 1959; *Gerschwind* 1961; *Bentzel et al.* 1964; *Eisenberg* 1966).

The increase in urinary calcium could be caused by: increase in calcium filtered load, decrease in tubular resorption rate, or by an association of both mechanisms.

The first suggestion could result from an increase in plasma filtrable calcium concentration and/or an increase in glomerular filtration rate.

The first of these possibilities can be readily discarded by the absence of

any significant change in plasma calcium levels during the experimental periods (Table 1). In addition, we have demonstrated that dexamethasone therapy is associated with a slight but significant reduction in dialyzable serum calcium (*Wajchenberg*, to be published).

An increase in glomerular filtration rate could explain the hypercalciuria after dexamethasone treatment. In this sense, there are many references in the literature which indicate that there is an increase in glomerular filtration rate after the administration of glucocorticoids (*Ingbar et al.* 1951; *Levitt & Bader* 1951; *Liddle* 1959; *Soffer* 1960). If this were the only mechanism to explain the increase in renal excretion of calcium there should not be any change in urinary specific activity as was observed (Table 6), since there is no reason to postulate a different renal handling of stable and radioactive calcium after dexamethasone.

On the other hand, the studies of *Laake* (1960), *Gardner et al.* (1963) and those of *Walser & Robinson* (1963) have indicated that tubular reabsorption of calcium is reduced by corticoid therapy. However, the acceptance of this mechanism still has to meet the above mentioned criticism in connection with the increase in urine specific activity. The same can be said for the suggestion of an association of changes of filtration rate and tubular reabsorption to explain the hypercalciuria.

In the patients with hypercortisonism, in both its natural and iatrogenic forms, the decrease in tubular calcium reabsorption (*Laake* 1960; *Gardner et al.* 1963; *Walser & Robinson* 1963) would make available for excretion, ionized calcium of high specific activity which could be excreted as such and/or due to the availability of anions, particularly phosphates, greatly increased in the urine of such a patient (Table 1), as complexes with high specific activity. Anyway there would be an increase in urine specific activity after the induction of hypercortisonism, independent of the effect on glomerular filtration rate. Such an increase in urinary specific activity would necessarily be accompanied by a decrease in systemic calcium specific activity, as was in fact observed in every case (Table 6).

2. *Effect on intestinal absorption of calcium*

Treatment with dexamethasone, in the normal subjects, did not alter total faecal calcium, despite changes in endogenous faecal calcium (decrease) and in unabsorbed dietary calcium (increase) – Table 5 – which compensated each other. The increase in unabsorbed dietary calcium was obviously dependent on the decrease in fractional absorption rate ($\alpha$). However, the finding of a decrease in $Ca_Fe$ (and being the non-absorbed fraction of Ca d. j.) must lead to the conclusion, in the presence of reduced $\alpha$, of a primary decrease in Ca d. j.

A decrease in both $\alpha$ and Ca d. j. suggest that dexamethasone induces a decrease in the rate of calcium transfer across the intestinal wall, in both directions (from the lumen to plasma and vice-versa) more intensely from the mucosal cell to the lumen (secretion).

Similar observations, limited, however, to intestinal absorption, are mentioned by *Miravet & Landron* (personal communication).

More information is available from animal experiments (*Harrison & Harrison* 1960; *Milhaud et al.* 1960; *Kimberg et al.* 1961; *Garrett et al.* 1962).

*3. Effect on the constants of compartmental analysis*

From the several parameters studied (Tables 3 and 4) only the transfer of calcium from bone to the exchangeable pool (R) varied significantly (increase) after dexamethasone. Quantitative microradiographic observations of bone remodeling in Cushing's syndrome by *Riggs et al.* (1966), the studies done in rabbits by *Storey* (1961) and *Eisenberg*'s (1966) strontium kinetic studies are in agreement with the present data.

The response of bone deposition rate ($Ro_{13}$) was variable, increasing in two patients but not in the others. The striking reduction in $Ro_{13}$, in the case of Cushing's syndrome, in the active phase as compared to the remission stage, was also reported by *Eisenberg & Gordan* (1961) and *Eisenberg* (1964) and by *Lafferty & Pearson* (1964) in cases of long standing Cushing's syndrome and severe osteoporosis. The quantitative microradiographic studies of *Riggs et al.* (1966) mentioned above lead to similar conclusions.

The longer persistence of high steroid levels in cases of Cushing's syndrome in relation to the acute administration of pharmacological dose of dexamethasone could explain the discrepancies mentioned in both situations.

From all this discussion it can be said that the acute administration of dexamethasone, in normal subjects, in the dose and for the period of time that it was used in the present study, induces an increase in urinary calcium with higher specific activity, a decrease in intestinal calcium absorption and accelerated bone resorption. The question now arises whether all these effects are directly dependent on multiple hormonal actions or whether they are secondary to some primary site of action of steroid on calcium:

a) A primary action of the glucocorticoid on calcium in the kidney would induce renal wasting of calcium and should, for the preservation of a normal plasma calcium level, induce an increase in bone resorption (secondary hyperparathyroidism) but there would be no reason for simultaneous reduction in intestinal calcium absorption (*Jackson & Dencaster* 1959).

b) Constant total faecal calcium due to opposite and compensated changes in intestinal absorption and endogenous faecal excretion is not compatible with the suggestion that the primary steroid effect is to depress intestinal calcium ab-

sorption, the renal and bone changes being induced by a secondary hyperparathyroidism in order to maintain normal plasma calcium levels (*Collins et al.* 1962).

c) The primary site of action of the steroid could be the skeleton, producing an increased bone resorption with a tendency for hypercalcaemia (*Grollman* 1954) which would inhibit parathyroid hormone production. This condition of secondary hypoparathyroidism would reduce intestinal absorption and renal tubular calcium resorption, as would be expected from the known actions of the parathyroid hormone (*Rasmussen* 1961) to maintain normal plasma concentration of calcium ions. In support of this concept, *Eliel et al.* (1965) found that the administration of glucocorticoids reduces the parathyroid hormone-like activity in the urine due to a decreased secretion.

However, all findings cannot be explained by this mechanism, as the decrease in endogenous faecal calcium with constant total faecal calcium excretion and the reduction in renal tubular reabsorption of calcium induced by dexamethasone in a patient with idiopathic hypoparathyroidism (*Wajchenberg et al.* 1965).

The possible role of thyrocalcitonin cannot be discussed at present; it is known that it protects against hypercalcaemia (*Pechet et al.* 1967) but the need for, the extent and the degree of this protection is unknown.

Therefore, our data suggest that the increased resorption is also the result of a direct effect of the glucocorticoids on bone.

Despite the fact that some of the effects of dexamethasone on calcium metabolism could be mediated by changes in parathyroid hormone secretion rate, the results of our studies have to be interpreted as showing a direct effect of this agent on the kidney, gut and skeleton.

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