PLASMA CONCENTRATIONS OF RADIOCALCIUM AFTER ORAL ADMINISTRATION, AND THEIR RELATIONSHIP TO ABSORPTION

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(Received 23 June 1969)

SUMMARY

1. Different radioisotopes of calcium were administered orally and intravenously to a series of patients, some normal and others with diseases that might affect calcium absorption or metabolism. Concentrations of the two isotopes in plasma were determined at intervals. A mathematical procedure was developed for deriving from these measurements the total absorption of the oral dose and also the absorption/time relationship.

2. The correlation between absorption and plasma concentration of the oral dose at various times after administration was studied and found to be very good, the 2 hr plasma level giving the best correlation, particularly when account was taken of the patient's weight.

3. The effect of variations in the disappearance curve of the injected isotope upon the shape of the plasma appearance curve of the oral isotope was examined. It is concluded that for the majority of cases, when absorption is not greatly delayed, and the exchange processes determining the shape of the disappearance curve are not markedly abnormal, 2 hr plasma concentration gives a good indication of absorption. When abnormalities in these processes are present, only the double isotope method gives accurate results.

4. The results were applied to examine the validity of other published methods of analysis. It was found that plasma concentrations of orally administered radiocalcium alone can give useful information about absorption, but not about plasma clearance.

Measurement of plasma levels of radioactive calcium after oral administration was introduced by Bhandarkar et al. (1961) to provide an estimate of calcium absorption. After considering serial measurements they decided that the 2 hr level gave a useful index of absorption and compared this with absorption as measured by metabolic balance studies. Other authors have used different times, and more recently the whole of the early part of the plasma concentration
curve has once again been employed to illustrate absorption (Avioli et al., 1965; Caniggia, Gennari & Cesari, 1965). These methods, however, do not measure the contribution of loss of endogeneous calcium in the faeces to calcium balance, and double isotope methods have been introduced to overcome this difficulty (Tothill & Dellipiani, 1965; De Grazia et al., 1965; Dellipiani, 1967). Such measurements have provided an opportunity to study the plasma concentration curves in relation to absorption, and the results of such a study are reported here. In addition a method of analysis has been developed which derives the relationship between absorption and time after administration. This in turn may help with the interpretation of other plasma concentration curves.

METHODS

For most of the tests 10 μCi of $^{45}$Ca as chloride were added to a glass of milk (200 ml containing approximately 250 mg calcium) and given to the patients after an overnight fast. At the same time 10 μCi of $[^{47}$Ca]chloride without added carrier was injected intravenously. For one group of twelve patients the oral dose was administered without any added carrier, the total amount of calcium present being less than 1 mg. For a few patients the isotopes were reversed, the $^{47}$Ca being given orally. In these cases the $^{45}$Ca dose was reduced to 3 μCi. Samples of blood were withdrawn at intervals up to 24 hr and urine and stools collected for 6 days. Forty-eight patients were studied, including a group of controls and patients with renal failure, primary malabsorptive disease, osteomalacia and some who had undergone gastric surgery. The diagnosis of primary malabsorptive disease was based on methods described elsewhere (Girdwood et al., 1966).

Plasma was assayed for $^{47}$Ca by well scintillation counting. Liquid scintillation counting was used to measure the $^{45}$Ca after $^{47}$Ca and the daughter $^{47}$Sc had decayed. Two methods of sample incorporation were used. In the first, calcium was precipitated from 5 ml plasma as the oxalate, which was dissolved in hydrochloric acid and blended with the phosphor using ethanol. Recovery was measured by $^{47}$Ca counting. The second method used 2 ml of plasma which was blended directly with a toluene-based phosphor, using 1.5 M hyamine hydrochloride. For $^{45}$Ca determinations in urine, calcium precipitation was from 50 ml aliquots. All results are expressed as percentages of the administered dose per litre of plasma.

Calculations

Two typical sets of results are shown in Fig. 1, representing oral administrations with and without carrier calcium. It will be seen that there is a very rapid initial fall in the plasma concentration of the intravenously administered isotope as the calcium moves out of the vascular compartment. The orally administered isotope usually has appeared in the plasma at 15 min and the concentration then rises to a peak 1–4 hr later.

Calculation of absorption and its variation with time

The absorption per unit time is clearly a function of time $a(t)$ and it is this function and its integral that we wish to determine. Each fraction of absorbed calcium is subject to dispersion according to another function $d(t)$, and the observed plasma concentration of oral radioactive calcium derives from the interaction of these two functions. The function $d(t)$ can be derived from the measurements of the plasma concentration of the injected isotope. It is very well
represented by a power law, as was observed by Anderson, Tomlinson & Osborn (1962). Thus, \( C = At^{-k} \), where \( C \) is the concentration at time \( t \), and \( A \) and \( k \) are constants. However, the function \( a(t) \) does not follow any consistent mathematical form and the deconvolution has to be performed numerically. Consider the amount, \( x \), entering from the gastrointestinal tract in a given time interval \( T \). It is useful to assume that \( x \) is absorbed instantaneously at a time \( fT \) before the end of the period, giving a plasma concentration at the end of the period equal to that expected if the same quantity entered the circulation at a constant rate throughout the period. \( fT \) then constitutes an equivalent dispersion time, and for short enough values of \( T \) can be considered as \( T/2 \). However, as \( T \) is lengthened, \( fT \) becomes less than \( T/2 \), due to the marked curvature of the plasma disappearance curve of injected material.

At the end of the first period \( T \), the observed plasma concentration of orally administered isotope \( (O_T) \) results from the proportion of the administered dose entering in that period, \( x_1 \), subject to dispersion for a time \( fT \). Then \( O_T = x_1 I_{fT} \), where \( I_{fT} \) is the proportional concentration remaining in the plasma at a time \( fT \) after intravenous injection, from which \( x_1 \) can be derived. Similarly after a time \( 2T \), the observed concentration \( O_{2T} = x_1 I_{T+fT} + x_2 I_{fT} \), giving \( x_2 \). The analysis is extended to the determination of \( x_3, x_4 \ldots \), until the amount entering in a given period \( T \) is found to be negligible.

The shorter the period \( T \), the more faithful is the representation of the absorption pattern, but the more tedious the calculation. The interval chosen in this study was \( T = 30 \) min, and then it was found that, on average, \( fT = 10 \) min. A few calculations were carried out with \( T = 10 \) min and the results compared with those obtained for \( T = 30 \) min. The differences were quite negligible, both in the absorption patterns and in the values of final absorption.

The results may be plotted as a histogram of \( x \) against time, showing most clearly when absorption rate is a maximum, or as an integrated curve of the total entry by a particular time (Fig. 2). The final figure for absorption is given by \( \Sigma x \) as \( t \to \infty \), and is the height of the plateau of the integral curve.

A close approximation to the absorption may be obtained by considering the ratio of isotope concentrations with an appropriate adjustment of time scale. The peak absorption rate after the oral dose had been administered in milk was at times varying from \( \frac{1}{2} \) to 2 hr after administration, and at this time nearly half the absorption had taken place. It can therefore be considered, without introducing much error, that absorption takes place instantaneously at the time of maximum absorption rate. Because of the shape of the plasma disappearance curve, the concentration ratio of the two isotopes in plasma will vary with the time after entry into the bloodstream, the longer this time the closer the ratio being to that of the amounts entering. However, the concentrations of activity are lower for the later specimens, and so accuracy is lost by extending the period of observations.

The plasma disappearance curve may be used to assess the error introduced by using the isotope concentration ratio to estimate absorption. Using the mean value of 0.30 for the exponent \( k \) in the power law equation, an interval of 1 hr between injections gives an error of 5% if concentrations are compared at 8 hr, and less than 2% for all times after 12 hr. For a 2 hr delay, the error is 8% at 8 hr, 6% at 12 hr and 3% at 24 hr. If, therefore, the ratios are determined from the later parts of the plasma concentration curves, with the adjustment of time scale, an accurate measure of absorption is obtained. The validity of this argument was demonstrated by the good agreement between the values of absorption obtained in this way and those from the previously described analysis.
If urine is used as a sample material it may not be so easy to fractionate collections. In addition, because of the very variable urinary concentration of calcium it would be necessary to determine this concentration on each sample and plot specific activity of each isotope against time. De Grazia et al. (1965) attempted to overcome this problem by administering the oral isotope 2 hr before the intravenous injection, because they found that the peak concentration in plasma occurred at about that time. It should be noted that this peak does not correspond with the maximum absorption rate, and that the time of the latter varies. However, when 24 hr samples of urine are used the error introduced is small. We have preferred to use simultaneous administrations, and estimate that the use of isotope ratios without adjustments of time scale in single samples of urine or plasma after 12 hr leads to an error in the calculated absorption of less than 10%.

RESULTS

The relationships between total absorption, endogenous faecal calcium loss and clinical condition will be considered in another publication. The results presented here stem directly from the analytical methods described.

Results of calculations of absorption rate

Typical absorption/time curves are plotted in Fig. 2 on both a differential and an integral basis. Fig. 2(a) refers to a case in which the oral dose was administered in milk and is calculated from the data in Fig. 1(a). Fig. 2(b) corresponds to Fig. 1(b) and in this case the dose was given without carrier. After carrier-free administration, with two exceptions the maximum absorption took place in the first $\frac{1}{2}$ hr. The time of maximum absorption rate was rather more variable after the dose was given in milk. In six cases it occurred in the first $\frac{1}{2}$ hr, in ten during the second $\frac{1}{2}$ hr, in nine during the third $\frac{1}{2}$ hr, in four during the fourth $\frac{1}{2}$ hr and in three cases at a later time. The seven patients with renal failure had a slightly later average time of maximum absorption rate than the overall mean, but otherwise there did not seem to be any correlation with clinical condition.

Plasma levels related to absorption

Since we have measurements of absorption by the double isotope method, together with complete plasma concentration curves, the values of the latter at various times after oral administration may be correlated with absorption. The time most commonly used for assessment of absorption is 2 hr after administration, and these results are shown in Fig. 3. Carrier-free administrations are shown as open circles, those with milk as dots. It is seen that the correlation between absorption and plasma concentration is good, the correlation coefficient being 0.91.

As the rate of absorption is different when administration is without added carrier from that when milk is given, it might be expected that the relationship between plasma concentration
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Fig. 1. Variation of plasma concentration of radiocalcium with time after administration, (a) with added carrier, (b) without.

Fig. 2. Variation of absorption of radiocalcium from the gastrointestinal tract with time, (a) administration with added carrier, (b) without.
Fig. 3. Plasma concentration of radiocalcium 2 hr after oral administration related to absorption. Closed circles, administrations with milk; open circles, no added carrier.

Fig. 4. Absorption related to 2 hr plasma concentration multiplied by patient's weight. Closed circles, administration with milk; open circles, no added carrier.
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and absorption would be different for the two groups. However, calculation of the regression lines shows no appreciable differences of slope or intercepts for the two hour concentration. The results are also shown in Fig. 4.

Comparisons of the 1 hr and 4 hr plasma levels with absorption were also made. Considering the combined measurements from all patients, the equations of the regression lines are:

\[ C_1 W = 2.92A - 26 \quad r = 0.91 \]
\[ C_2 W = 2.95A - 17 \quad r = 0.95 \]
\[ C_4 W = 2.17A - 3 \quad r = 0.89 \]

where \( C_1 \) is the plasma concentration at 1 hr in \% per litre, \( W \) is the patient’s weight in kg, \( A \) is the absorption in \% of the administered dose and \( r \) is the correlation coefficient.

The best correlation is obtained at 2 hr, although the other times are not much inferior. The effect of delayed absorption is most marked at 1 hr, as might be expected. The intercept of the regression line on the absorption axis is at 9\%. The lower slope of the 4 hr regression line is a consequence of the plasma concentration curve having passed its peak by that time in the majority of cases.

**DISCUSSION**

**Patterns of absorption and clearance**

Nordin et al. (1968) have attempted to calculate calcium absorption rate from plasma radioactivity, using a very simple model and method of analysis. All their deductions were based on the plasma concentrations of the orally administered radiocalcium, as they made no measurements of plasma clearance rate. They assumed that during the first 2 hr after administration

![Fig. 5. \( A_o - A_t \) plotted against \( t \), where \( A_t \) is the amount absorbed by the time, \( t \), after administration, (a) with milk, (b) without added carrier.](image)
calcium is absorbed at a constant fractional rate and cleared from the plasma at another constant fractional rate. As we have measurements of the clearance rate, and have deduced the pattern of absorption, it is interesting to apply the formulae of Nordin et al. (1968) to the same data to see how faithfully they represent the true situation.

If the fractional rate of absorption is \( k_1 \), then the amount that has been absorbed at a time \( t \) is \( 1 - e^{-k_1 t} \). If we let this equal \( A_t \), we can investigate the assumption of a constant rate by plotting \( A_\infty - A_t \) against \( t \) on semilogarithmic paper, when a straight line should be obtained. This has been done for all our data, using the analysis described to determine \( A_t \). It is found that the assumption is quite a reasonable one, provided that fractions of the radiocalcium finally absorbed are considered, rather than fractions of the administered dose. The general equation proposed by Nordin et al. (1968) does not take this point into account.

Two typical curves are illustrated in Fig. 5. Curve (a) represents the cases when administration was carrier-free. In these cases \( k_1 \) was most nearly constant with time, did not vary greatly between patients and was relatively high. The mean value was 1.40/hr. The pattern of absorption expressed in this manner was more variable when administrations of radiocalcium were in milk. Sometimes \( k_1 \) was very nearly constant but more typically increased with time, as illustrated in curve (b), Fig. 5.

Clearly, \( k_1 \) can be used as an index of absorption rate, although its variation with time in many cases makes it necessary to define the time at which it is measured. A time of 1.5 hr after administration was adopted, and an examination made of the relationship between absorption rate and clinical condition. The results are illustrated in Fig. 6. Some of the patients with osteomalacia had also had gastrectomies or were suffering from malabsorption. These are included only in the osteomalacia column, with appropriate identifying symbols. If administrations with milk only are considered, the mean value of \( k_1 \) is lower for the gastrectomy, osteomalacia and particularly the renal failure cases than it is for the controls. However, the groups are rather
small and the evidence for slower absorption is only of reasonable significance for the group of patients in renal failure.

Parsons, Veal & Butterfield (1968) have suggested that osteoporotic patients exhibit a slower absorption pattern than normal. Only four such patients were included in our series, and $k_1$ and the time of maximum absorption rate were within the normal range. However, two of the administrations were without added carrier and the numbers are too small to draw any conclusions.

Although relatively few deviations from the normal range have been observed in this series, the method does offer the possibility of studying abnormal patterns. The results may also throw some light on sites of absorption.

The approximate form of calculation proposed by Nordin et al. (1968) leads to a value, $x$, of the fraction absorbed per hour. It is of interest to compare the values of $x$ calculated for our data by Nordin's method with those derived from our method of analysis (say $f_1$), confining the comparison to the first hour after administration, in view of the differences with time of the fractions absorbed per hour if these are expressed in relation to administered rather than absorbed dose. A good correlation is observed, the regression equation of $f_1$ upon $x$ being $f_1 = 1.25x + 0.034$, $r = 0.91$. However, an even better correlation ($r = 0.97$) is obtained if $f_1$ is compared with the total activity in the extracellular calcium pool as deduced from the patient's weight and the 1 hr plasma concentration of orally administered isotope. Of course, the latter value enters prominently into the calculation of $f_1$, as it does for $x$, so that a good correlation is to be expected. However, this finding suggests that the analytical methods of Nordin et al. (1968) add little to the knowledge of absorption rate that is not yielded by the 1 hr plasma concentration alone.

Nordin et al. (1968) also attempt to calculate the fractional clearance rate, expressed as $y$, the fraction removed per hour, and we can calculate $y$ for our data by their method and compare it with the experimentally determined clearance rates derived from the plasma concentrations of the injected isotope. It is obvious that the assumption of a constant fractional clearance rate is not really compatible with the observed power law expression of concentrations. The latter was followed remarkably faithfully in all our cases, from times as early as 5 min after injection. If time, $t$, is expressed in hours, the exponent, $k$, of the power law equation represents the fractional clearance rate at 1 hr, and the rate at other times is $k/t$. Therefore, even over a period of 2 hr there is a considerable variation of fractional clearance rate. When $y$ calculated from our results was compared with the observed value of $k$, no correlation was found. The mean value of $y$ was 0.43/hr SEM 0.22, while the mean of $k$ was 0.30 ± 0.07. In fact, a much closer approximation to the true clearance rate would be obtained in the great majority of cases by assuming a value of 0.30 than by attempting a calculation of $y$ based on the concentration of orally administered radiocalcium. It may be concluded that the method of Nordin et al. (1968) does not yield any useful information about removal rate.

As can be seen from the above figures, there is not a large variation in $k$ between patients. The maximum value observed was 0.43/hr and the minimum 0.22, apart from one patient with hypercalciuria who gave a value of only 0.08. There did not seem to be any correlation with clinical condition although some of the groups were rather small.

**Effect of disappearance rate on plasma levels of oral isotope**

Since the variation of plasma concentration of the orally administered isotope depends on the
pattern of absorption and also on the disappearance of the absorbed material from the plasma, it is of interest to examine the effect of variations in the latter in order to assess the relationship between the quantity absorbed and the plasma concentration at various times. The factor $A$ in the power law equation is related to the early volume of distribution of the injected dose and the effect of this factor is largely taken into account by incorporating the weight of the patient as a multiplying factor when comparing plasma levels with absorption.

The effects of variations in the exponent, $k$, can perhaps best be illustrated by an example. A typical absorption curve as deduced by the method described is shown in Fig. 7, curve (a). In this case $k$ was 0.37, near the upper end of the range, and the operation of such a disappearance curve on curve (a) gives curve (b), which is, of course, also the experimentally determined concentration/time curve. If the same absorption pattern is taken, but a disappearance curve with $k = 0.22$, at the lower end of the range, is assumed, normalized at 0.1 hr, the result is curve (c). Obviously, the slower disappearance of absorbed calcium leads to plasma levels being higher, and the time of maximum concentration is somewhat later. The proportional change of concentration increases with time after administration, being 1.12 at 1 hr, 1.24 at 2 hr and 1.49 at 4 hr. These factors represent the sort of error due to variations of slope of the disappearance curve that might apply to the deduction of absorption from single values of plasma concentration. As the two values of $k$ chosen for the illustration are near the ends of the normal range, the error arising from the lack of knowledge of $k$ is not likely to be large unless $k$ is really abnormal, for example in conditions when bone exchange processes are markedly slowed. The earlier plasma concentrations are less affected by variation in $k$ than the later ones, and this may help to explain why the correlation between absorption and plasma concentration is better at 2 hr than 4 hr.

![Fig. 7. Effect of different rates of radiocalcium disappearance from plasma upon the plasma appearance curves of an oral dose with a given time pattern of absorption: (a) absorption pattern, (b) plasma concentration curve with $k = 0.37$ and (c) with $k = 0.22$.](image-url)
Avioli et al. (1965) concluded that plasma levels of radiocalcium during the first 4 hr after oral administration are determined primarily by absorption and are little affected by the exchangeable calcium pool. Their evidence was rather indirect and the foregoing analysis provides a more quantitative conclusion.

Comparison with other published results

The majority of previous studies have compared plasma levels at various times after oral administration with calcium absorption as determined by metabolic balance studies. The correlation has not been close, which is not surprising, as the radioisotope technique is concerned with the absorption of a single dose in a defined chemical form by a fasting patient, whereas balance studies measure the mean absorption, over a long period, of dietary calcium in a variety of forms.

A better correlation \( r = 0.89 \) was obtained by Avioli et al. (1965), who compared the 1 hr plasma \( ^{47}\text{Ca} \) activity with absorption as assessed from the cumulative 6 day faecal \( ^{47}\text{Ca} \). On the other hand, Parsons et al. (1968) comparing the 2 hr plasma concentration with absorption determined from faecal excretion, found only a moderate correlation coefficient of 0.54, although they deduced that the distribution was bi-modal, with osteoporotic patients representing a different population from the remaining patients.

The faecal method of measuring absorption neglects endogenous faecal loss, but our measurements support the conclusion of Avioli et al. (1965) that this factor may, in general, be neglected. We found that a mean value of 8% of an injected dose is found in a 6 day stool collection. A more important source of error in many cases is likely to be incomplete collection of stools.

There do not seem to have been any previous comparisons between absorption as measured by a double isotope technique and plasma concentrations of the oral dose. The correlation found in our results was better than that reported by other workers, particularly when the patient's weight was taken into account. It is suggested that this is due, at least in part, to the greater accuracy of this technique in measuring absorption, particularly as the correlation was maintained when isotope ratios from late plasma or urine samples were used to assess absorption, in place of the analysis of the early plasma concentrations. No independent measurements of absorption were available in our series. The slopes of the regression lines relating plasma concentration to absorption in our series are within the rather wide range reported by other workers. Using balance techniques, Nordin (1968) found that net absorption could be obtained approximately by multiplying the 2 hr plasma concentration by the patient's weight in kg \( \times 0.15 \). Our results show that the true absorption of a single dose is obtained by using a factor of 0.28.

The analytical methods described in this paper allow us to examine the reasonableness of the conclusions drawn from other published data. For example, Caniggia et al. (1965) report on the intestinal absorption of \( ^{45}\text{Ca} \) in stone-forming patients. They present full plasma activity curves for six stone formers and the range of results found in five normals. In the first group, plasma activity rises more rapidly to a higher and earlier peak than in the normal cases. The levels then fall more rapidly until there is little difference between the two groups at 2 and 4 hr. In view of the correlation already reported between plasma levels at these times and absorption measured by the double isotope method, it seems necessary to examine further the contention of Caniggia et al. (1965) that the results demonstrate increased absorption in stone forming patients.

We have no knowledge of the plasma disappearance rate of injected radiocalcium in these
cases, but can consider the effect of an identical disappearance curve on the mean results of the two groups. The absorption pattern was calculated for each group using the mean plasma concentration curve of injected calcium from our own series. The cumulative absorption figures for the means of Caniggia's two groups were 55% for the stone formers and 52% for the normals. The small difference hardly justifies the conclusion reached. The deduced absorption pattern confirms what is fairly clear from inspection of the original findings, namely that absorption takes place earlier in the stone-forming patients, but that it persists longer in the normals.

It may be, of course, that there is a significant difference between the plasma disappearance curves of injected or absorbed radiocalcium in the two groups. The earlier peak of the plasma concentration curve in the stone-forming patients could be due, at least in part, to a steeper disappearance curve. If this were so, then the observed concentration curves would also correspond to a higher value of absorption. There is, however, not sufficient evidence to support this hypothesis.

Conclusions

The methods outlined in this paper allow the determination of absorption and its variation with time. No attempt has been made to relate total absorption to particular diseases. Clearly various conditions may be expected to affect absorption, particularly when patients are considered in groups. Absorption is also markedly influenced by the conditions of administration. However, for the range of conditions used and the patients examined in this study, the relationship between absorption and plasma concentration does not seem to have been affected in the great majority of cases. The analysis has shown that the two factors which might affect this relationship are delayed absorption and a plasma disappearance rate which differs markedly from the normal. Under these circumstances only the double isotope method can give an accurate measure of absorption from plasma concentration determinations.

The data presented show that while plasma levels of orally administered radiocalcium on their own can give some information about absorption, they do not at present give useful information concerning the clearance of the absorbed material.

It is likely that the double isotope method, coupled with the analytical techniques described, could be applied to the investigation of the absorption of other substances.

ACKNOWLEDGMENTS

We are grateful to Dr J. D. Simpson and Dr J. M. Bone for their co-operation and to Mrs Sandra Dickson and Miss Carole Deans for technical assistance. We are also indebted to those physicians and surgeons, particularly Professor R. H. Girdwood, Dr J. S. Robson and Mr J. Chalmers, who allowed their patients to be investigated.

REFERENCES

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