LXXIV. INTERACTION OF VITAMIN D AND DIETARY FACTORS IN THE HEALING OF RICKETS IN RATS.

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(*Received February 23rd, 1934.*)

RICKETS produced in rats by a high calcium-low phosphorus diet may be healed either by the administration of vitamin D or by the addition of a source of phosphorus to the diet. The estimation of the influence of small additions of sources of phosphorus or of other related changes in composition of the diet has, however, received much less attention than the relation between the degree of healing and the dose of vitamin D. It is of considerable practical importance to find some means of expressing the antirachitic effect of such dietary factors, both alone and in conjunction with vitamin D, more especially in those cases where a measure is to be made of the vitamin D content of foodstuffs containing phosphorus compounds. From the theoretical point of view, the discovery of such a relation would throw some light on the synergism of vitamin D and phosphorus compounds.

The exact form of the effect of added phosphorus compounds on the healing produced by vitamin D may be complicated. However, two simple alternative approximations suggest themselves. The antirachitic activity might be additive, *i.e.* equivalent to that of an additional dose of vitamin D, or it might be multiplicative, *i.e.* multiplying the effect of doses of vitamin D actually administered. Key and Morgan [1932] found that by changing the ratio of calcium to phosphorus from 4 : 1 to 2 : 1 in a certain rachitogenic diet, a healing effect was produced equal to that of a dose of 0·7 International Unit (I.U.) of vitamin D with the original diet. Again, Lecoq and Villette [1933, 2] found the curative dose of vitamin D for rachitic rats on the Randoin-Lecoq diet to be 20 I.U. incorporated in 100 g. of diet. The curative doses of various phosphates had previously been found [Lecoq and Villuis, 1932; Lecoq and Villette, 1933, 1], and they now proceeded to assign to these compounds potencies in antirachitic units. This form of expressing the effect of changes in mineral composition of the diet is convenient and is justifiable when vitamin D is not administered simultaneously; but it is unjustifiable to assume, without further experiment, any particular relation of the combined effect to the effects of the two influences separately. A certain ambiguity may arise in this connection, and it is important to make quite clear the distinction between the sum of the effects, measured separately, of two influences and the effect of their sum when applied simultaneously. The expression of both dietary changes and doses of vitamin D in antirachitic units would seem to imply that these two influences should be summed in order to calculate their combined effect. Both groups of workers do, in fact, conclude that the effect of simultaneous administration of phosphate and of vitamin D will be the sum of their separate effects.

When partial healing is produced by administration of vitamin D to rats on a rachitogenic diet it is commonly recognised that although the general form of the curve relating dose to healing effect must be **S**-shaped, the central portion is approximately logarithmic. Thus for a considerable range of dose the degree of healing is proportional to the logarithm of the dose. This relation has been found for radiographic measurements of healing by Everse and Van Niekerk [1931] and by Bourdillon *et al.* [1931], for the line test by R. S. Morgan [1932], for growth response by Coward *et al.* [1932], and also for measurements by the prophylactic method using A/R ratios [Hume *et al.*, 1932] or a radiographic scale [Bourdillon and Bruce, 1932]. In the case of the radiographic scale used by Bourdillon *et al.* [1931], this relation is expressed by the equation

$$\log_{10} D_2 - \log_{10} D_1 = \frac{(n_2 - n_1)\log_{10} 2}{2},$$

where n_1 and n_2 are the scale numbers of litter-mates which have received doses D_1 and D_2. With a homogeneous stock of rats kept under uniform conditions, the effect of animal variation is reduced to a minimum, and the mean degree of healing, n, in a group of rats will be fairly closely proportional to the logarithm of the dose, $\log D$. The graphical relation between n and $\log D$ in an ideal case will be a straight line, *e.g.* (a) in Fig. 1.

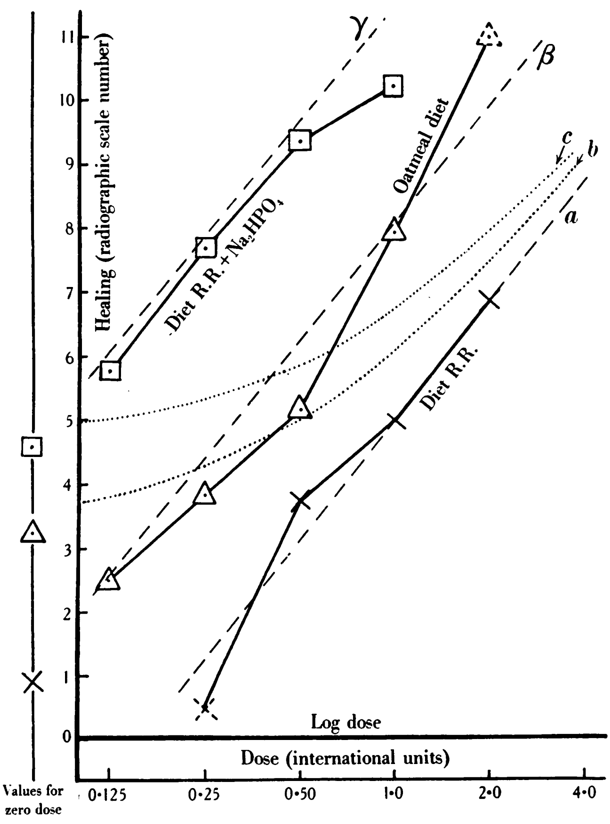

Fig. 1. Comparison of experimental and theoretical relations between log dose and healing.

——— Lines joining experimental points.
— — — Ideal line for diet R.R. (a), and lines calculated from this on multiplicative basis (β and γ).
.... Curves calculated on additive basis (b and c).

If, now, an alteration in the diet is equivalent to the addition of a dose of vitamin D, say d, the relation becomes

$$n \propto \log (D+d),$$

and the relation between the logarithm of the actual dose given and the scale number is no longer linear, *cf.* curves (b) and (c) in Fig. 1, which deviate considerably from the original line at low values of D, but approach it at high values.

If, however, an alteration in the diet has the effect of multiplying the dose by a factor ϕ, the relation becomes

$$n \propto \log \phi D = \log \phi + \log D.$$

The relation is still linear, but the line is shifted a distance along the n axis corresponding to $\log \phi$, and remains parallel with the line for the original diet, as is shown in Fig. 1, curves β and γ. At higher doses there is a clear and increasing difference between the values given by additive and multiplicative constants, corresponding to the increasing difference between the sum of these two constants and their product. The curves b and c in Fig. 1 show that a constant addition to the actual dose given has a varying effect with different doses, whilst the lines β and γ in Fig. 1 show that multiplication of the dose by a constant factor produces a constant addition to the effect, increasing the healing by the same number of degrees on the scale with any dose.

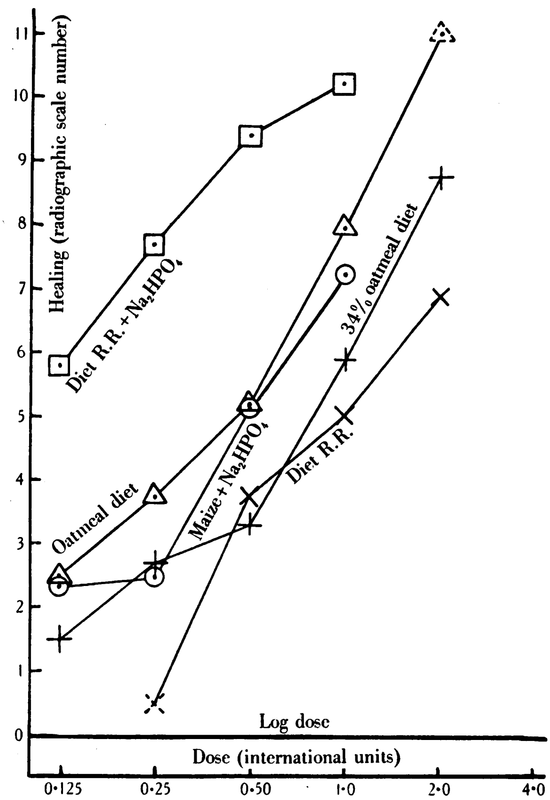

Fig. 2. Relation between log dose and healing. Experimental data for five diets.

In the course of the investigation described in the next paper [Bruce and Callow, 1934] we had occasion to compare quantitatively the calcifying powers of diets containing either different cereals or different forms of phosphorus, and we had to settle the question of how these differences were to be expressed. In Fig. 2 is shown the result of plotting degree of healing against logarithm of the dose with five diets for which suitable data are available. The picture corresponds reasonably well with a series of parallel lines, and there is no resemblance whatever to a series of convergent curves such as would be required by additive influences. Confirmation is afforded by Fig. 1, in which data obtained for the two least rachitogenic diets (oatmeal and R.R. + Na_2HPO_4) are plotted and compared with curves calculated on the basis of additive constants (b and c) or multiplicative factors (β and γ) applied to the standard rachitogenic diet (R.R.). The evidence is decisively in favour of multiplicative factors for both diets.

Experimental.

Data.

Full details of the technique and of the diets used are given in the next paper [Bruce and Callow, 1934]. The data from which the graphs in Figs. 1 and 2 have been constructed are given in Table I.

Table I.

Description of diet	Dose (International Units)	Healing Mean radio-graphic scale no.	No. of rats
R.R. (routine rachitogenic diet: 20% maize, 48% white flour, 32% basal mixture)	Nil	0·9	4
	(0·25	0·5	2)
	0·5	3·76	15
	1·0	5·02	45
	2·0	6·9	5
34% oatmeal (a mixture of equal parts of R.R. (above) and oatmeal diet (*cf.* below))	Nil	0·33	3
	0·125	1·5	3
	0·25	2·7	3
	0·5	3·3	6
	1·0	5·9	5
	2·0	8·75	4
Maize + Na_2HPO_4 (68% maize, 32% basal mixture, 2·46 g. Na_2HPO_4 (anhydr.) per kg.)	Nil	0·5	4
	0·125	2·33	3
	0·25	2·5	7
	0·5	5·14	18
	1·0	7·22	11
Oatmeal (68% oatmeal, 32% basal mixture)	Nil	3·23	58
	0·125	2·5	4
	0·25	3·85	17
	0·5	5·19	50
	1·0	7·95	17
	(2·0	11·0	2)
R.R. + Na_2HPO_4 (R.R. (above) + 8 g. Na_2HPO_4 (anhydr.) per kg.)	Nil	4·57	31
	0·125	5·79	19
	0·25	7·7	41
	0·5	9·38	13
	1·0	10·25	4

Calculation of additive constants.

A straight line is drawn through the points for diet R.R. in Fig. 1. The doses, as given by this line, corresponding to the scale numbers given by rats on oatmeal diet and on R.R. + Na_2HPO_4 diet with no administered dose, determine the values of d for these diets. The values are $d=0{\cdot}51$ unit for the oatmeal diet, and

$d = 0{\cdot}84$ unit for the R.R. + Na_2HPO_4 diet. The theoretical curves (*b*) and (*c*) are then plotted, substituting, at values of the logarithm of the actual dose, values for the scale number corresponding to an assumed dose, $(D+d)$.

Calculation of multiplicative constants.

The values of the multiplicative factor are derived from a series of comparisons of litter mates on diet R.R. and the other two diets, which is described in detail in the paper following this. The relation for the radiographic scale, *viz.*

$$\log_{10} D_2 - \log_{10} D_1 = \frac{(n_2 - n_1)\log_{10} 2}{2}$$

becomes $$D_2/D_1 = \text{antilog}\,[(n_2 - n_1) \times 0{\cdot}1505].$$

In the case of two diets A and B, the value of D_2/D_1 which is found is an "apparent dose ratio," a_A/a_B. If the ratio of the doses actually administered is d_A/d_B, then the apparent dose ratio is given by

$$\frac{a_A}{a_B} = {}_A\phi_B \times \frac{d_A}{d_B},$$

where ${}_A\phi_B$ is the multiplicative factor for diet A with respect to diet B, and

$${}_A\phi_B = \frac{a_A}{a_B} \times \frac{d_B}{d_A}.$$

Referred to diet R.R., the value for ϕ for the oatmeal diet, determined from the mean of a number of direct comparisons, is 3·2, whilst for the R.R. + Na_2HPO_4 diet, $\phi = 8{\cdot}35$.

Summary and Conclusions.

When vitamin D is administered to rachitic rats on a diet containing excess of calcium, the influence of increasing or altering the source of phosphorus may be expressed as a factor multiplying the dose of vitamin D. Expression of the dietary influence as the addition of a certain number of antirachitic units to the actual dose of vitamin D gives figures which are not concordant with experimental results. The combined antirachitic effect of changes in the mineral composition of a diet and of simultaneous administration of vitamin D is thus greater than the effect of the sum of the two acting separately. It is actually more nearly proportional to the product of two factors representing these antirachitic influences.

It is a pleasure to record our thanks to Dr R. B. Bourdillon for his helpful criticism in the course of preparing this work for publication.

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