THE PHYSIOLOGY OF THE MOUTH

by

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PREFACE

The aim of this book is to provide an account of certain topics in physiology and biochemistry which are of importance in dental surgery but which tend to be neglected, or given a non-dental emphasis, in the standard textbooks. It has been assumed that the reader has already acquired a general knowledge of physiology and biochemistry.

Although the book has been written primarily for undergraduate students it is realized that with certain subjects on which general reviews are not readily available (for example calcification and saliva) the treatment has been somewhat fuller than they require. It is hoped by this means that the book may be of interest to post-graduate workers in dental surgery and to physiologists interested in this and allied fields.

No attempt has been made to give full references but those included are either reviews, papers with extensive bibliographies, or papers which are of outstanding interest in themselves. From these sources most of the statements in the book can be traced to their origin. The text has been written almost entirely from the original papers.

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CONTENTS

CHAPTER I

CALCIUM AND PHOSPHORUS METABOLISM

A somewhat detailed account of the metabolism of calcium and phosphorus may seem a curious beginning to a book on the Physiology of the Mouth. However, for a full understanding of the formation of the teeth, and the factors which may affect them, it is necessary to consider the general metabolism of these minerals. Consequently, this first chapter has been included to give a more comprehensive picture of certain aspects of their metabolism than is readily obtainable from most standard textbooks on physiology. It must be emphasized that in this chapter the metabolism of calcium and phosphorus in the body as a whole is discussed without special reference to the teeth. The influence of diet, hormones and body metabolism on the teeth is considered in later chapters.

Factors Controlling the Absorption of Calcium and Phosphorus

Although the major foodstuffs are absorbed almost completely from the gut, the proportion of certain inorganic ions absorbed may be small, since they readily form insoluble salts. Calcium and phosphate are among the ions which are poorly absorbed, and many factors influence the process, some favouring and others hindering it. Since the formation of insoluble calcium phosphate is the main (but not the only) factor in preventing absorption, it is clear that the absorption of neither ion can be considered alone.

The Measurement of Calcium Absorption

Before discussing the factors controlling absorption the method of studying this question will be briefly described.

The absorption is measured by subtracting the amount of calcium and phosphorus in the faeces (as found by analysis) from the calcium and phosphorus of food (found by analysis of an aliquot of each article of food eaten). This difference represents *net* absorption. It is important to realize that faecal calcium is not merely unabsorbed food calcium, since it is believed that the daily output of the digestive juices contains about 1 g. of calcium which is about the same as the amount in one day's intake of a good diet, and this calcium is treated in the same way as the calcium in the food and may, or may not, be reabsorbed. It is for this reason that under especially unfavourable conditions, there may be more calcium in the faeces than in the food. The question of the excretion of calcium through the large intestine is discussed on p. 27.

The factors which affect calcium and phosphorus absorption are as follows:

(1) *Vitamin D and Calcium Absorption*

There is good evidence from 'calcium balance' experiments (described more fully on p. 13) with intact animals which suggests that one of the functions of vitamin D is to favour the absorption of calcium. In many experiments on the mode of action of vitamin D, it has been impossible to decide whether the primary action has been on calcium or on phosphate because improved absorption of one, by reducing the tendency of calcium phosphate to precipitate, indirectly favours the absorption of the other. The experiments of Nicolaysen overcame this difficulty by studying, in normal and vitamin D deficient rats, the absorption of calcium from diets containing no phosphate, and vice versa. The results suggested that vitamin D increased calcium absorption and had no direct effect on the absorption of phosphate. This conclusion was confirmed by experiments in isolated loops of intestine by the following method. In anaesthetized, fasting rats, loops of the intestine were sealed off from the rest of the gut by ligatures with as little interference with their blood supply as possible. A solution of calcium chloride, or sodium phosphate or other salt as required, containing a known amount of salt was injected into the loop which was then returned to the abdomen. After five, and in some experiments twenty-four hours, the animals were killed and the amount of calcium or phosphate remaining in the isolated loop was estimated chemically. Since the ligatures prevented any mechanical movement of fluid into or out of the loop, any changes in the calcium or phosphate concentration must have been by absorption through the gut wall into the circulation. By this technique, it was very clearly shown that the rate of absorption of calcium was lower in the vitamin D deficient rats than in the normal controls. The effect of the vitamin was specific for calcium, as the rates of absorption of neither phosphate (both inorganic and combined with sugar) nor sugars nor sodium sulphate differed in the normal and deficient rats. A criticism of these experiments, using loops of intestine of fasting rats, is the unnatural condition of the gut, for example, the absence of food and digestive juices; but they did confirm the results of other suggestive, but less decisive, experiments on intact animals. As stated above, under normal conditions after a mixed meal, when both calcium and phosphorus are present in the intestine, reduced absorption of calcium by vitamin D deficiency would raise the concentration of calcium in the intestine and favour the precipitation of calcium phosphate, thus leading secondarily to a reduced absorption of phosphate.

The means by which the absorption of calcium may be selectively increased by vitamin D is still unknown, although attempts have been made to explain it on the basis of pH changes in the gut. It is known that in human vitamin D deficiency, the faeces are more alkaline than is usual and direct measurements of the pH of the intestinal contents in deficient rats have shown a tendency for the ileum and large intestine to be slightly nearer the alkaline side, than in normal rats. The effect seems too small to be important, and could not, in any case, account for Nicolaysen's results since in the absence of phosphate, calcium chloride will remain soluble over a wide range of pH. It seems possible that the tendency towards greater alkalinity in the intestines of vitamin D deficient animals is a *result* of impaired calcium absorption, leading to the formation of larger quantities of sparingly soluble alkaline calcium salts.

The effect of vitamin D on the gut mucosa is of long duration and it takes several weeks on a vitamin D deficient diet before the reduced absorption of calcium is markedly reduced.

Many experiments have been described, both in man and in animals, in which the addition of vitamin D to a diet has had little or no effect on calcium absorption. These negative results are probably accounted for by the fact that only a very small amount of the vitamin is required to exert the maximal effect on calcium absorption, and the provision of more cannot, of course, increase an already maximal effect. If the diet is rich in calcium, or in substances which favour its absorption, then vitamin D has a smaller effect than it has with diets low in these constituents.

Other possible actions of vitamin D are described later (p. 10).

(2) *Effect of pH of the Intestine*

The acid of the gastric juice dissolves most calcium salts and inorganic phosphates likely to be in the diet, but precipitation may occur in the small intestine as the contents become neutralized. It has often been assumed that the absorption of calcium only occurs in the upper part of the small intestine where the pH is still well on the acid side, but this view may now require revision (see p. 8). On the other hand, there is good evidence that if the gut is made more acid (e.g. if milk is acidified with HCl before ingestion), a higher proportion of its calcium is absorbed. As will be noted later, many factors which favour calcium absorption may possibly do so by increasing the acidity of the gut.

(3) *The Amount of Dietary Calcium and Phosphorus*

It follows from the Law of Mass Action that excess of, say, calcium in the gut will tend to precipitate phosphate and it might be expected therefore to reduce its absorption. In animal experiments with very great disparity between the calcium and phosphorus of the diet (i.e. with very high or very low Ca/P ratios) absorption has been found to be impaired. The effects of the addition of moderate amounts of phosphate to the diets of *human* experimental subjects have been varied and have not always caused a reduction in calcium absorption. These variations cannot be explained with certainty but it is possible that if other factors in calcium absorption are unfavourable, then phosphate may reduce absorption but the effect may be nullified if other factors (see below) are favourable.

The Ca/P ratio is particularly important in the rat, a species in which vitamin D deficiency alone does not produce rickets, but requires also either a high or low Ca/P ratio in the diet. For this reason, it is necessary to be cautious in applying to the human subject the results of experiments on calcium metabolism in rats. If the amounts of calcium and phosphorus in the diet are high, the Ca/P ratio becomes less important in controlling absorption.

As the levels of calcium and phosphorus in a diet are increased the amounts absorbed also increase, but in different proportions. For example, in one experiment in rats, when the calcium and phosphorus in the diet was almost trebled, the amount absorbed was only about doubled. There is clearly a limit, therefore, to the beneficial effects obtainable from increasing the dietary level of these minerals.

(4) *Phytic Acid*

Sir Edward Mellanby observed in 1921 in experiments on puppies, that if oatmeal or whole wheat were included in a diet low in vitamin D or calcium, the severity of the rickets was increased. The anti-calcifying factor present in these foods became known as 'toxamin', since it seemed antagonistic to vitamin D. Later work showed that phytic acid (the hexaphosphate of inositol) was the active substance and it reduced calcium absorption by forming an insoluble salt with calcium, probably containing five atoms of calcium, although, if magnesium ions are also present, a mixed calcium and magnesium salt is formed whose composition depends on the relative concentration of the two ions. Phytic acid is present in seeds where it may act as an inert store of phosphate to be broken down to inositol and inorganic phosphate by the enzyme phytase, which is either present in the grain (as in wheat) or formed during germination (oats). In cereals, the phytic acid occurs mostly in the outer part of the grain: consequently brown flour (technically known as 'high extraction flour' and usually containing 92 per cent of the whole wheat) contains much more phytic acid than does white flour ('low extraction flour' usually containing about 70 per cent of the constituents of the whole wheat).

The importance of phytic acid in the human dietary has been demonstrated by several groups of workers who have shown that absorption of calcium by human subjects from diets containing brown flour is less than from white flour. The most comprehensive work has been carried out by McCance and Widdowson, who, in addition to comparing calcium, magnesium and phosphate absorption on diets containing 70 per cent extraction flour with those on 92 per cent extraction flour, have also shown that the addition of phytic acid to white flour and its removal by enzymes from brown flour, respectively lowered and raised the absorption of calcium and magnesium. The periods on each diet were usually three weeks, which is an important point because many experiments of this type have been vitiated by using periods which were too short. The phytic acid does not merely immobilize the calcium and magnesium in the cereal, but may also precipitate these ions from other constituents of the diet, or, if these are low, from the digestive juices. If the latter occurs, the faeces contain more calcium than the diet and the subject is losing instead of absorbing calcium from the gut. The effect of phytic acid is quantitative and can therefore be neutralized by adding sufficient extra calcium to the diet to combine with it, a procedure adopted by the Ministry of Food when the National Loaf (85 per cent extraction rate) was introduced in Great Britain in 1942. In Dublin, the introduction of a high extraction flour without fortification by calcium was followed by a rise in the incidence of rickets.

The experiments just mentioned tend to exaggerate the effect of phytic acid in two ways, first, since wheat and yeast both contain phytase, which may be active in splitting phytic acid during the rising of bread, bread contains considerably less phytic acid than does flour. In the human experiments mentioned, this destruction of phytic acid was avoided. Secondly, the calcium content of the diet was deliberately kept low, so that the amount precipitated was a substantial proportion of the total calcium present.

An important question at once arises. Many human diets do in fact contain much phytic acid and little calcium, without apparently causing the disastrous interference with calcium metabolism which might be expected. It has been suggested that eventually adaptation to a high phytic acid diet can be made and some experiments which extended over nineteen weeks lend support to this view. During the first few weeks on a fairly high phytic acid diet, the expected fall in absorption of calcium occurred, but more and more calcium was absorbed in succeeding weeks throughout the experiment. There is no entirely satisfactory explanation of how this possible adaptation to phytic acid is achieved but it may be linked to an increase in intestinal phytase. Possibly the continued presence of phytic acid in the gut, encourages the growth of bacteria containing phytase.

The importance of phytic acid cannot be regarded as settled, but it may be concluded tentatively that if the diet contains this substance regularly it has little influence on calcium absorption. If it is suddenly introduced or increased in a diet, there may be a marked temporary reduction in calcium absorption.

Effect of Phytate on Phosphate Absorption

Several workers have found that under certain conditions, about 50 per cent of the phosphorus of phytic acid may be absorbed in the human gut. This fact indicates that a phytase, probably of bacterial origin, must be present in the gut. The amount of calcium in the gut is one of the most important factors deciding how much phytic acid is broken down; the higher the calcium concentration, the more will insoluble phytate be precipitated, and the less will it be available in a soluble form for splitting by phytase.

Some experiments have suggested that vitamin D favours the breakdown of phytic acid. Although a direct effect has not been disproved, these observations are equally well explained by supposing that vitamin D operates by favouring calcium absorption and thus indirectly favouring phytase action, as explained above.

(5) *Effect of Oxalates*

Oxalate is another acid radical present in certain foods which makes calcium unavailable. Several vegetables contain traces of it, but spinach and rhubarb leaves contain relatively high concentrations. The free oxalate in the edible portions of these foods may precipitate significant amounts of calcium from other constituents of the diet or from the digestive juices, and in addition, the whole of the calcium contained in these foods is unavailable. In rhubarb leaves (but not the stalks), free oxalic acid occurs in toxic quantities. At least one death resulted from the eating of stewed rhubarb leaves as a vegetable during the food shortage in 1917.

(6) *Influence of Fat on Calcium Absorption*

No simple answer can yet be given to the question of whether fat increases or decreases the absorption of calcium from a normal diet.

Some experiments have indicated that raising the fat content of rats' diets increases faecal calcium (that is, reduces calcium absorption) and this has been explained on the basis of the formation of insoluble calcium soaps. In other experiments, it has been found that the absorption of calcium was favoured by increasing the fat of the diet. In rats, dietary fat appears to lower slightly the pH of the gut and this would favour calcium absorption. Most of these observations have been made with unnatural and unbalanced diets, however.

It is possible that the type of fat fed, and variations in the other constituents of the diet, determine whether fat increases or decreases calcium absorption. For example, when fed to rats, the calcium soap of oleic acid has been found to be much better absorbed (90 per cent) than was the calcium soap of stearic acid (45 per cent). Some experiments on rats suggest that if fat is present at a low level in the diet (5 per cent) it may favour calcium absorption whereas a larger proportion of fat (25 per cent) may reduce it.

(7) *Effect of Protein and Amino-Acids*

Lehmann and Pollak, in the course of some experiments on the effect of amino-acids on enzyme action, observed that alkaline suspensions of certain sparingly soluble salts of calcium and magnesium became clear when amino-acid solutions were added. This suggested the possibility that the proteins of the diet might increase the solubility, and hence the absorption, of calcium salts from the intestine. Experiments in which calcium and magnesium absorption were compared in human subjects on diets as similar as possible except that one contained about three times as much protein as the other, showed that a considerable increase in calcium absorption occurred with a high protein diet.

This work may explain several seemingly anomalous observations, e.g. the highly calcified bones and teeth of the Eskimos who live on a low calcium, high protein diet, and the anti-rachitic action of lean meat and skimmed milk (both of which contain little vitamin D) observed in dogs by Mellanby.

It must be emphasized that this effect of protein may allow calcium to be soluble in, and therefore absorbed from, an alkaline medium and the assumption that has usually been made in the past, that the contents of the gut must be acid for calcium absorption to occur, must now be questioned. Nevertheless, it is still true to say that acidity in the gut *favours* calcium absorption but it may not be essential unless the diet is very low in protein.

(8) *Effect of Carbohydrate*

When calcium absorption has been studied from diets containing different carbohydrates, it has been found with several species of animal, that lactose favours calcium absorption and bone calcification. It has been shown by direct measurements in rats receiving a high lactose diet that the intestines become more acid than in the control animals, which in turn would favour calcium absorption. Other constituents of the diet may modify this effect, however; on a high meat diet, for example, lactose had only a small effect on the pH of the intestine. It has been suggested (though proof is lacking) that lactose is digested and absorbed more slowly than other disaccharides, and by lingering in the gut encourages the development of acid-producing organisms. It has also been shown that calcium and magnesium form unionized complexes with lactose and these reactions might play a part in keeping calcium in solution and therefore available for absorption.

The effect of lactose on calcium metabolism has not been adequately investigated in human subjects, but in one experiment on young boys, calcium retention was improved by high lactose feeding. This was caused by smaller urinary excretion, however, and absorption seemed unchanged. This suggests that lactose may have other important influences on calcium metabolism in addition to its possible effect on absorption.

(9) *Seasonal and unexplained effects*

In the course of their prolonged experiments on human subjects, McCance and Widdowson observed that in three out of six subjects large seasonal variations occurred in the absorption of calcium, but not of magnesium or phosphate. The absorption was least in February and March and greatest in July and August, suggesting at first that variations in sunlight and consequently in the amount of vitamin D synthesized by the skin and present in the food, might be responsible. The administration of large doses of vitamin D in March had little effect on the calcium absorption, however, which makes it unlikely that the reduced absorption in winter is caused by vitamin D deficiency. McCance and Widdowson suggested that a seasonal variation in the responsiveness of the tissues to vitamin D might explain their observation. As the absorption changed with the seasons, so did the urinary excretion, that is, there was no seasonal change in the amounts of calcium retained by the body.

An even more remarkable change was observed in the absorption of calcium by all of five subjects who were studied continuously over the period July 1940 to July 1942. In 1941 the absorption of calcium was less complete than in 1940 and 1942. No explanation has been suggested, nor has a year to year variation been reported by others, but very few experiments have continued for sufficiently long periods to test this point.

(10) *Personal Variation*

Many workers have observed that the amount of calcium absorbed varies from individual to individual even under apparently identical conditions. Some people consistently absorb a higher proportion of their dietary calcium than others, and although the addition of, say, phytic acid to the diet lowers calcium absorption in all, the amount of change in different subjects may be very different. For example, one individual may absorb 50 per cent of the calcium in a diet without phytic acid and 30 per cent if phytic acid is added; another may absorb 20 per cent and none respectively from diets without and with phytic acid.

The causes and significance of these changes are not understood. It is not known whether good absorbers have a metabolic need for larger amounts of calcium, nor is there evidence to suggest that poor absorbers suffer from defectively calcified skeletons.

(11) *The Effect of Body Stores of Calcium*

An experiment on rats has suggested that the amount of calcium stored in the body is a factor which may influence calcium absorption. One of two groups of comparable rats was fed a diet containing 0.15 per cent calcium for four weeks, the other group received the same diet except that the calcium level was 0.8 per cent. The average calcium retained by each rat in the two groups was 191 and 570 mg. of calcium respectively and thus the bodily store of calcium was much greater in the second group. During a second period which lasted five weeks, both groups received 0.4 per cent calcium in their diets. The group with low calcium store absorbed about 900 mg. calcium per rat and the high calcium group absorbed only 600 mg. per rat. This experiment raises the possibility that some of the variations found in calcium absorption in man may be due to previous nutritional history, and some observations on human subjects have supported that view. The mechanism of this effect is unknown, but there is evidence that vitamin D is necessary for it.

(12) *Effect of Pregnancy and Growth*

There is evidence from human metabolic studies which suggests that the percentage of dietary calcium absorbed increases in the later months of pregnancy. Although there is an increase in urinary excretion also, it is smaller than the increase in absorption so there is a net gain during pregnancy estimated at about 45 g., of which half is in the foetus and the rest is stored by the mother's skeleton, presumably, as a reserve for lactation. This implies that the metabolic needs of the body in pregnancy are influencing calcium absorption, probably through the action of sex hormones.

Growing animals tend to absorb a higher proportion of their dietary calcium than do adults.

The Fundamental Action of Vitamin D

We have seen that vitamin D probably plays a direct part in the absorption of calcium (and only indirectly of phosphorus) from the intestine and the question arises as to whether this is the sole means by which this vitamin influences calcium and phosphorus metabolism, or whether it has additional functions in the calcified tissues or elsewhere. The question is not finally decided, but there is a good deal of evidence which taken together strongly suggests that vitamin D does have some action in tissues other than the intestinal mucosa. The evidence is as follows:

(1) In human rickets, the blood calcium is usually normal, but the blood

phosphorus is invariably low. If vitamin D is concerned only with calcium absorption, it is difficult to see why blood phosphorus alone should be low. As will be described more fully in chapter III, when slices of rachitic bone are incubated in suitable solutions they are found to lay down calcium salts. Rachitic tissues require higher concentrations of calcium and phosphate in the incubating solutions for calcification to occur than do normal tissues, thus showing that in rickets there is some local defect in the calcifying tissue and that changes in blood composition are not the only factors involved.

(2) If salts containing radio-active phosphorus or calcium are *injected* into rachitic rats, some of which are untreated and others are partly cured by receiving vitamin D, it is found that the treated animals lay down 50 per cent more radio-active element in the bones than do the untreated. Since absorption from the gut can play no obvious part in the metabolism of injected material, the conclusion is that the greater uptake by bone must be due to some effect either in the bone itself or in some other tissue concerned in calcium and phosphorus metabolism. This experiment does not necessarily prove that vitamin D has caused increased *laying down of additional salts* in the bone, as the result could equally well be explained by increased *replacement* of salts without any actual addition. The fact remains, however, that vitamin D appears to alter bone metabolism independently of intestinal absorption.

(3) A number of experiments have been published, in which for various reasons, vitamin D has not influenced calcium absorption but has, nevertheless, improved the structure of the bone. As already mentioned, the effectiveness of vitamin D in increasing calcium absorption depends on several factors including the other constituents of the diet. For example, in rats on a high protein diet, vitamin D was found not to influence calcium retention but did improve the histological structure of bone and teeth.

(4) Studies on the excretion of phosphate by the kidneys of rachitic dogs before and after receiving large doses of vitamin D have shown that the vitamin causes a reduction in the amount of phosphate excreted, by increasing the amount reabsorbed by the kidney tubules. If these observations are confirmed with smaller, more physiological doses of vitamin D, they would explain the low blood phosphate, and indirectly possibly some of the other changes, characteristic of rickets.

(5) It is known that very high doses of vitamin D have effects almost exactly the reverse of those produced by small doses (see next section). Recent experiments have clearly proved that these effects are at least