CXCIX. RICKETS IN CHICKENS, WITH SPECIAL REFERENCE TO ITS NATURE AND PATHOGENESIS.

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MUCH has been written on "leg weakness" in chickens, a subject of varied aetiology and pathology although the term is often employed as synonymous with rickets. One is not here concerned with those varieties included under this designation which are associated with lack of vitamin B, absence of some factor in milk [*vide* Hogan *et al.*, 1925; 1928; Norris *et al.*, 1930; 1931; Bethke *et al.*, 1931], neurolymphomatosis or pellagra. Perosis or "slipped-tendon," however, from the special circumstances in regard to Ca and P metabolism surrounding its genesis, merits detailed remark. There is a voluminous literature on this subject. Amongst recent papers are those by Titus [1932]; Holmes *et al.* [1933]; Parkhurst and MacMurray [1933]; Branion [1933] and many others. Two characteristics of the condition are worthy of note: firstly, that cod-liver oil is powerless to prevent or cure it; and secondly, that nothing abnormal is to be found on histological examination of the bones. As regards aetiology, it is almost universally agreed that the most commonly associated precursor of the condition is the presence of large amounts of $\text{Ca}_3(\text{PO}_4)_2$, as bone meal, in the ration. This point will be discussed later, but meantime it may be mentioned that elsewhere [McGowan, 1933, 2] evidence has been adduced to show that metabolism of Ca and P is much less efficiently carried on when these elements are fed in chemical union with one another. The alternative, of course, is to feed the Ca as the salt of some other acid radical than P_2O_5 , while the P may be supplied in a "delayed-action" form as food-phosphorus which requires to be broken up into simpler chemical form by digestion before it is absorbed. In this case it will happen that the Ca is for the most part dissolved out and absorbed before the P is in a form to unite with it. The prevention of perosis [Titus, 1932] by the administration of rice bran, rich in organic P of the "delayed action" type, lends support to this view. Melby [1933], by statistical analysis of experimental results obtained by various authors, also showed that a high P content¹ of the ration was associated with a high percentage occurrence of perosis.

With regard to rickets, the main subject of this communication, some preliminary considerations require emphasis. It is a singular fact that histological examination of the bones has played no part whatever as a routine procedure and check in experiments on the production of rickets in chickens by means of various diets. Indeed, there are very few contributions at all in the literature dealing with the morphological appearances in the bones, and such as exist are mainly for the purpose of settling some minor disputed point, or *ad hoc* and of the nature of investigations in pathology. One mentions, more or less in the

¹ Excess P had been given as $\text{Ca}_3(\text{PO}_4)_2$.

former context, the work of Hart *et al.* [1925], Hughes [1924], Pappenheimer and Dunn [1925] and Hughes and Titus [1926]. Pappenheimer and Dunn [1925] maintained that the condition present in the bones of chickens fed on the usual "rickets"-producing ration (that of Hart [1924])¹ is not rickets and would seem effectively to have disposed of the allegations to the contrary of the other investigators mentioned. Pappenheimer and Dunn, however, while describing the condition present do not venture on its classification on a pathological basis. At a later date, Nonidez [1928] made a more definite contribution to this subject. He pointed out that the bones of the chick on hatching were in a much earlier stage of development than those of the mammal at birth. He alleged that their bones reached the stage of those of the new-born mammal only after five weeks of post-hatching life, a view which will afterwards be shown to be incorrect. On a certain special diet, his experimental chickens failed to grow and at six weeks most of them were dead, while their bones, he stated, showed the changes already described by Pappenheimer and Dunn. The addition of semi-solid buttermilk to this ration, together with exposure of the birds to sunlight, permitted the chickens to live longer and, at the same time, to exhibit better growth and bone development, though these apparently were not as yet optimum². Chickens were now reared for the first 4 weeks on this modified ration together with exposure to sunlight, and thereafter, the semisolid buttermilk having been withdrawn from the ration, were kept indoors and away from sunlight. Birds so treated, he alleged, developed rickets, and this of a type comparable with that of mammals: but it must not be forgotten that in his diagnosis of rickets he stresses the existence of enlargement of the ends of the bones, beading of the ribs, *etc.*, as an essential feature. The presence of such lesions in his later experiments as compared with his earlier, can, however, be attributed to the greater age to which the chickens of the former lived; while it will be shown later that such enlargements occur as frequently and as characteristically in osteoporosis as in rickets, and that sunlight and vitamin D have equal effects in preventing both. Other points in his claims are, moreover, not quite clear. Thus a ration which, when fed from hatching onwards, produced (as Nonidez admits) Pappenheimer and Dunn's non-rickety condition, is alleged to be able to bring about true rickets when fed to healthy chickens over six weeks of age, a claim which appears not to be fully substantiated by the illustration (Fig. 1) accompanying his paper. This photograph depicts an appearance more in keeping with a modified osteoporosis, although it is admitted that a band, which might be interpreted as a rachitic metaphysis, is present and is designated as such in the text. He finds, in addition, enlargements of the parathyroids and a lowered Ca content of the blood of his birds, conditions more in harmony with the existence of osteoporosis, as will afterwards be pointed out. That sunlight prevented and cured the condition elicited by him in his experiments has no significance either way, as it has as great a beneficial influence on certain types of osteoporosis as on rickets.

The histological investigations, therefore, which have been undertaken in the sphere of the Ca and P metabolism of the chicken would seem, so far as they show anything, to have demonstrated the existence of osteoporosis, while nothing of the nature of rickets has with certainty been disclosed. Moreover, they were undertaken without idea of their serving as a check on the results

¹ From personal observations, the condition produced in chickens fed on this ration (yellow maize 97, CaCO₃ 2, NaCl 1 part), preventable and curable by cod-liver oil, is osteoporosis—not rickets.

² See footnote, page 1507, in reference to this point.

obtained in feeding experiments. The only check that has been consistently and largely applied for this purpose is an estimation of the total ash or the Ca and P in the bones. Unfortunately such a procedure is exceptionally open to criticism, apart from the fact that several authors, such as St John *et al.* [1933] and Bethke and Record [1934], have impugned the accuracy of the test as such. Osteoporosis, a condition differing in every other particular from rickets, resembles it only in having a low ash content in the bones and the swelling of the ends of them already mentioned. Thus, from the experimental results presented below it will be seen that while the tibiae of normal chickens of group BD had 51 % ash at six weeks of age, those of the osteoporotic chickens of groups AD and B at the same age had respectively 36.05 and 41.2 % and those of the rickety chickens of group C 27.8 %. Similar figures were obtained in these groups at 2, 3, 4 and 5 weeks of age¹. The significance of such results is apparent, and as a sequel to such considerations, the value of much of the previous work on rickets in chickens is incapable of being assessed owing to the lack of certitude regarding the exact condition, osteoporosis or rickets, under observation.

Criticism can also be applied to the voluminous literature that has arisen around the alleged importance in nutrition of the chicken of the ratio of Ca to P in the ration. This is quite apart from the obvious fact that the same ratio may exist between very different absolute quantities of Ca and P. In addition, evidence has been adduced elsewhere [McGowan, 1933, 2] to show that ratio, *per se*, is of no importance and that, apart from a real absolute deficiency of Ca or P in the ration, the only matter of moment in this connection is the percentage content of Ca in the ration in relation to its power of neutralising the HCl of the gastric juice and thereby setting in motion a train of injurious consequences.

EXPERIMENTAL.

The present investigation deals in the main with the histological changes present in the bones and the implications of such in chickens reared on diets of a nature such as had previously been alleged to produce or prevent rickets, and forms the culmination of a series of observations carried out on this subject since 1926.

The chickens employed were 3-day old White Leghorns. They were reared in brooders inside a house and away from sunlight. The basal diet was as follows:

Yellow maize	75	parts
Dried skim milk	10	„
Dried yeast	15	„
Sodium chloride	0.5	„

The six groups (15 birds in each²) employed and the CaO and P₂O₅ contents of the various rations are given below:

Group		CaO %	P ₂ O ₅ %
A	Basal	0.24	1.49
AD	Basal + 1 % cod-liver oil	0.24	1.49
B	Basal + 1.2 % CaCO ₃	0.91	1.48
BD	Basal + 1.2 % CaCO ₃ + 1 % cod-liver oil	0.91	1.48
C	Basal + 6 % CaCO ₃	3.6	1.48
CD	Basal + 6 % CaCO ₃ + 1 % cod-liver oil	3.6	1.48

¹ The lowest ash percentage (22.6 %) was obtained in the case of an osteoporotic chicken (group AD) placed at 6 weeks into group C (rachitic) for 3 weeks.

² Owing to their greater rate of growth, males suffered more severely than the females.

At intervals of a week the chickens were weighed individually, and specimens from each group were removed and killed for the purpose of examination of the bones histologically and with regard to their ash content. The weights of the chickens at 6 weeks, exhibiting as they do the same relative difference between the various groups as had been obtained in the previous four weighings, is first submitted. The figures for group A, basal ration group, are omitted as the chickens in this group were all dead before the expiry of the third week.

Average weight of chickens (g.) of each group at 6 weeks:

Group BD	333	(normal bones)
„ CD	269	„ „
„ C	213	(rachitic bones)
„ B	186	(osteoporotic bones)
„ AD	107	„ „

The average weight in group BD—333 g.—is up to the standard of growth for this race of fowl under ideal conditions of food and surroundings (*vide* Charles and Kandel [1928]; also personal observation). The series of body weights given here will be seen by comparison with the preceding table to run parallel with the amount of Ca actually available for ultimate metabolism in the various groups and, in this connection, attention may be particularly directed to the very divergent results as regards mortality, weight and growth in group A (basal) as compared with group AD (basal + cod-liver oil), where the very low Ca in the basal diet is seen to be made in some degree more available in the second group by means of vitamin D. As will also be seen from a comparison of the size of the bone sections in the various groups at 6 weeks of age (*vide* Plate IX), the difference in size of the body as a whole is paralleled by a similar divergence in the size of the bones. Deficiency of Ca (with ample P present) would appear, therefore, to bring about lack of tissue growth and an atrophy and to produce in the bones osteoporosis, as will be seen more fully immediately; while overabundant Ca (with consequent deficiency of P occurring simultaneously) allows growth, possibly of an exuberant nature, to proceed, which in the bony tissues remains immature and gives rise to rickets, as will be discussed shortly.

As a detailed description of the histological appearances present in the bones is attached to the illustrations accompanying this paper, only the broad general findings will be dealt with here. From the examination made at the end of six weeks, they may be summarised as follows:

- (1) Groups BD and CD (basal + CaCO_3 + cod-liver oil) bones normal (*vide* Plate IX, Figs. 3, 4)
- (2) Groups A (basal)
 AD (basal + cod-liver oil) osteoporosis (*vide* Plate IX, Figs. 5, 6)
 B (basal + 1.2 % CaCO_3)¹
- (3) Group C (basal + 6 % CaCO_3) rickets² (*vide* Plate IX, Fig. 7)

The same results were obtained in the bones from the various groups at the end of 2, 3, 4 and 5 weeks from the commencement of the experiment. Group BD, normal group, in contrast to the view expressed by Nonidez [1928] on this

¹ Somewhat similar osteoporosis with ample CaO in the diet and preventable by the addition of cod-liver oil has been noted in pigs [McGowan, 1933, 3].

² The appearances present here differ somewhat, though not in essentials, from those usually found in mammalian rickets, especially in sections of costo-sternal rib junctions. They are simpler in type in the present instance, which would seem to indicate, as the appearances do, that in the latter case complications have arisen owing to movement and fracture and the production of callus [*vide* McGowan, 1924].

subject (*vide supra*), showed the epiphyses fully formed and of "mammalian type at birth" at one week of age (Fig. 2)¹.

Chickens from groups AD and B (osteoporotic groups) and from group C (rickets) when placed at five weeks of age in group BD (normal) showed complete recovery and normal appearance of the bones in a week (Plate IX, Figs. 8, 9, 10). The recovery may actually have been more rapid since specimens for examination were taken only at the expiry of a week.

Chickens from group AD (osteoporotic group) transferred at 6 weeks of age to group C (rickets group) and kept there for 3 weeks showed then a mixture of osteoporosis and rickets, while the ash percentage of the bones (22.6 %) was the lowest obtained in the investigation.

Other findings of importance emerged. Thus, consistently in all the examinations at the various time intervals of a week, the parathyroids in groups A, AD and B (the osteoporotic groups) were found to be enlarged: while in groups BD and CD (the normal groups) and group C (the rickety group), they were normal in size. Doyle [1925], Nonidez and Goodale [1927], Bloom [1932] and Higgins and Sheard [1933] have remarked on the enlargement of the parathyroids in chickens in cases where, however, deprivation of vitamin D was regarded as the causal precursor: but it will be seen from the above classification that this enlargement of the parathyroids has relation rather to deficiency of available calcium in the body. Nonidez [1928] has indeed pointed out what he regards as a significant difference in the reactions produced by rickets in mammals as compared with chickens in that he found, in chickens affected with "rickets," a lowered Ca in the blood, a condition of matters, he remarks, not found in the mammalian disease. In this case, however, all the attendant circumstances indicate that the disease in his chickens was in reality osteoporosis and not rickets; and the results obtained in this experiment support this contention. It was found that, while the normal and rickety groups showed Ca 11-12 mg./100 ml. Ca in the blood-serum, the osteoporotic groups were quite definitely subnormal in this respect, having only 9.5 mg./100 ml. serum. Higgins and Sheard [1933] obtained similar low figures for the serum-Ca (8.55-6.38 mg./100 ml.) by feeding chickens on a diet containing dicalcium and tricalcium phosphates, although, as they remark, the calcium provided in the diet in this way was adequate, a point of interest in connection with the findings obtained in group B (basal + 1.2 % CaCO_3) in the present experiment. In their case too, the Ca was rendered available by the administration of cod-liver oil.

Enlargement of the bones, especially of the distal ends of the sternal and vertebral ribs and of their heads and tuberosities, was found to a comparable degree in the osteoporotic and rachitic groups, if due allowance be made for the age and growth of the individual bird. This is contrary to the view expressed by Nonidez (*vide supra*).

Observations were carried out on the point raised by Nonidez [1928] of the difference in histological appearance of the bone lesions according to whether the chickens were put on experiment at once on hatching or only after 5 weeks of post-hatching healthy growth. Chickens from groups BD and CD (normal with normal epiphyses) were, at the age of 5 weeks, transferred to group C (rachitic group) while, at the same time, others from the same normal groups were put into group B (osteoporotic group). The conditions produced in the bones, after a suitable lapse of time following such transference, were the same in type as

¹ This indicates that Nonidez's ration, even when fortified with dried buttermilk, was far from being a good one, and that indeed from the outset normal calcification did not occur in his chickens.

those obtained where birds, immediately on hatching, were placed in groups C and B, and were modified only to a very slight degree by the more developed condition of the epiphyses. The rachitic ration still produced unmistakably rachitic, and the osteoporotic ones, osteoporotic lesions (Plate IX, Figs. 11, 12).

DISCUSSION.

The results obtained in this experiment will be discussed in relation to the theory elaborated elsewhere regarding the nature of vitamin D action [McGowan *et al.*, 1931; McGowan, 1933, 1, 2, 4]. The essential action of vitamin D, according to this theory, is supposed to be two-fold in nature: firstly, the setting free of nascent phosphoric acid from the phospholipins of the tissues and, secondly, on account of this, the production of an acidotic tendency of the body, which, acting in the blood, prevents the precipitation of Ca as calcium phosphate and favours its circulation in soluble form as the chloride. This acid condition also favours the secretion of HCl in the gastric juice, with resultant benefit to the absorption of Ca and P.

The results obtained in group AD (basal + 1 % cod-liver oil) would appear to show that, of the two actions of vitamin D just referred to, the liberation of phosphoric acid is, as indeed one would expect, the more fundamental and important. In the group under survey, the Ca, present in deficient amount in the ration but presumably all absorbed, is, in contradistinction to what happens in group A, by the intervention of vitamin D made available and anchored in the body for further utilisation. The beneficial influence of vitamin D in this case, however, is circumscribed by Ca being present in too small quantity; hence the comparatively unfavourable results obtained in this group.

The results of the later groups show how markedly cod-liver oil, when Ca is present in adequate amount, can compensate for the unsuitable form of presentation (as CaCO_3) or over-abundant supply of this substance. Under favourable conditions of supply of Ca and P, as in milk, mammals can be reared without the aid of vitamin D when the percentage of CaO and P_2O_5 is as low as 0.4 [McGowan, 1933, 3]. Figures are thus forthcoming for a minimum concentration of these substances in the ration for healthy growth in mammals in the absence of vitamin D. Attempts have been made to arrive at similar figures for the rations of chickens; but the value of any so far obtained is discounted by the fact that vitamin D, in some form or other, was operative in every case at the same time.

The body of the chicken (differing in this respect markedly from the mammal), when hatched from the shell, owing to Ca during incubation being obtained only in sparse amounts from the egg-shell, is greatly lacking in Ca and its bones are in a markedly osteoporotic condition (Plate IX, Fig. 1). It is quite evident, therefore, that, in regard to Ca metabolism, the findings derived from mammalian studies cannot be transferred bodily to avian conditions. The distinction already pointed out by Nonidez [1928] in regard to the histology of the bones is concrete evidence of this.

While the ordinary diet of chickens (consisting as it does for the most part of grains) supplies abundant P_2O_5 and this in "delayed-action" form, it is quite otherwise with regard to calcium, which is notoriously lacking in such food. It is highly probable that the P_2O_5 requirements of the birds will be completely met from the food without recourse to vitamin D being required. Calcium, however, in some form or other, must be added to such a ration, and only further investigations directed to this point will be able to elucidate whether the addition of some certain Ca-salt, in an optimum amount alone and without the aid of

vitamin D, will suffice for healthy growth or whether it is essential that vitamin D be added at the same time. Observations already carried out in connection with this matter, show that Ca, added to the present basal experimental ration as calcium chloride or lactate in amount supplying as much as 1 % CaO, failed to prevent osteoporosis. At 4 weeks of age, the birds on this modified ration were only half the weight of birds of the same age in group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil (0.91 % CaO)), while their bones were in a condition of advanced osteoporosis.

The compensatory action of vitamin D, in cases where ample Ca has been added to the ration, is not only undeniable but considerable. In all likelihood, it depends in this case to a great extent on its acid-producing powers: but, of course, the fixing power on calcium of nascent phosphoric acid must not be lost sight of. As the circulating blood can hold only a limited amount of Ca in solution, if, under ordinary circumstances, large quantities of Ca (as CaCl_2) are absorbed rapidly from the food into the circulation, much of it will be excreted immediately in order that the blood composition, *qua* Ca, may be kept within normal limits; and in the alkalotic tendency produced by the administration of large amounts of CaCO_3 , the precipitation and consequent loss of Ca from the blood will be intensified. *Per contra*, an acidotic tendency due to vitamin D will favour its retention in the circulation. It will also render easier the secretion of acid in the gastric juice, with consequent better solution and absorption of Ca. Both these factors would seem to play an important rôle in assisting Ca metabolism and fixation in the body and in transforming the osteoporotic condition, present in group B for instance, into the normal one found in group BD.

The same factors would seem to be active in bringing about the more or less normal conditions present in group CD as contrasted with the rickety ones in group C. An important distinction, however, has to be made between the fundamental activities operative in the two sets of circumstances. In the set at present under discussion, the phosphoric acid-generating power of vitamin D would seem to play a relatively greater part¹ while the increased acidity of the stomach would seem to effect its purpose in a somewhat different way. In reference to the last point, the CaCO_3 , present in great excess, is rapidly rendered soluble by the more abundant HCl and removed by absorption into the blood, so that it is no longer able by its presence in the intestine to interfere with and render insoluble as $\text{Ca}_3(\text{PO}_4)_2$ the products of the "delayed-action" phosphorus when they are set free by digestion.

In the young fowl, therefore, osteoporosis on the one hand as the result of too little Ca² and rickets as the result of too much Ca on the other, are alike prevented by the presence of vitamin D. In all probability, this condition of matters arises from the osteoporosis with which the chicken starts life, coupled with the extensive Ca metabolism which consequently has to be carried through in a very short period of time to make up the leeway.

Less stringent conditions with regard to Ca exist in the early life of the young mammal, in which, under ordinary conditions, rickets (due to a relative deficiency of P) is the more common occurrence. Rickets, when it occurs in birds, is usually brought about artificially as the result of an endeavour to overcome, by generous administration, the initial failing of lack of Ca with its consequent osteoporosis; while in mammals, the condition is frequently initiated or, when

¹ By making phosphoric acid in a nascent form circulate more abundantly in the body and also by setting it free actually in the cells where calcification has to take place.

² Although ample Ca is present in the food.

already present, aggravated through a similar administration of calcium, based on a misunderstanding of its essential pathogenesis.

It is perhaps not without significance that several authors [Payne *et al.*, 1932; Titus, 1932; Parkhurst and MacMurray, 1933] have laid emphasis on the point that the administration of large amounts of CaCO_3 in the ration does not lead to perosis (rickets is not produced in these cases as vitamin D is administered at the same time), but that the feeding of large quantities of $\text{Ca}_3(\text{PO}_4)_2$ along with vitamin D, in an endeavour to prevent rickets, is very prone to do so. This would seem to indicate some recondite interference with phosphorus metabolism, possibly, as already suggested, related to the fact that Ca and P, when joined in chemical union, are relatively unsuited for tissue metabolism.

SUMMARY.

It is very unlikely, owing to the type of food usually given, that chickens will suffer from a deficiency of P_2O_5 but almost certain that Ca will be lacking.

This latter deficiency is exaggerated and aggravated by the osteoporotic condition of the body of the chicken on hatching, a circumstance which necessitates a very active Ca metabolism to meet it. Vitamin D has only a very slight influence in compensating for this lack of Ca.

In the usual amended diet of chickens, large quantities of Ca, generally as CaCO_3 , are incorporated but, owing to the alkalosis induced by such foods, the Ca is not absorbed and metabolised, and the condition of osteoporosis, already present, persists and is exaggerated.

It is doubtful whether, even if regard be had to the exact chemical combination of Ca given and the amount of it supplied, Ca in itself as such and without vitamin D could be administered so as to avoid this complication. This point can only be settled by further experimentation, which has already been commenced.

Administered in amounts larger than those associated with the appearance of osteoporosis, Ca, by interfering with the absorption and metabolism of P, produces rickets.

Vitamin D by its fundamental action, working along different lines in the two conditions just mentioned, prevents, on the one hand, the occurrence of osteoporosis, due to deficiency of available Ca, and, on the other, rickets, due to P deficiency.

An important precursor of the condition, perosis, is the feeding of large quantities of CaO and P_2O_5 in chemical union with one another.

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DESCRIPTION OF FIGURES ON PLATE IX.

In the preparation of the sections, the bones (lower ends of tibio-tarsus) were fixed in 5 % formalin for 24 hours and then cut with the freezing microtome. They were then treated by Von Kossa's silver nitrate method and mounted in glycerol. The sections are in the sagittal plane and the magnification (5 diameters) is the same in each case. There is thus afforded a rough means of comparison as regards growth between the various groups. Calcium deposits are stained deep black.

- Fig. 1. Tibia; newly hatched chicken showing *a*, epiphyseal cartilage; *b*, junction of epiphyseal cartilage with shaft cartilage; *c*, cylinder of "foetal" cartilage; *d*, slight deposit of calcium on surface of cartilage column and subperiosteally.
- Fig. 2. Tibia; chicken, group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil); epiphysis fully formed and of "new born mammalian" type after 1 week on this ration: 1 week old: showing *a*, epiphyseal cartilage; *b*, zone of hypertrophied cartilage cells well defined; *c*, spongiosa with numerous well calcified bony trabeculae enclosing normal marrow in their interstices—normal bone.
- Fig. 3. Tibia; chicken, group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil): 6 weeks old: as in Fig. 2—normal bone (lettering as in Fig. 2).
- Fig. 4. Tibia; chicken, group CD (basal + 6 % CaCO_3 + 1 % cod-liver oil): 6 weeks old: as in Fig. 2—normal bone (lettering as in Fig. 2); *d*, centre of calcification of epiphysis.
- Fig. 5. Tibia; chicken, group AD (basal + 1 % cod-liver oil): 6 weeks old, showing *a*, epiphyseal cartilage; *b*, absence of zone of hypertrophied cartilage cells; *c*, "foetal" cartilage (the darkish patches here are due to the presence of folds not to calcification); *d*, very sparse patchy deposit of calcium on free end of this cartilage and subperiosteally; entire absence of calcification elsewhere in this section (although in the actual specimen the background is colourless and without calcified areas, it has been found impossible fully to reproduce this in the photograph)—advanced osteoporosis—(owing to the persisting deficiency of calcium, the intraovular conservative mode of formation of supporting tissues survives to a great extent).
- Fig. 6. Tibia; chicken, group B (basal + 1.2 % CaCO_3): 6 weeks old: as in Fig. 5—advanced osteoporosis. The great difference between this specimen and Fig. 7 should be noted.
- Fig. 7. Tibia; chicken, group C (basal + 6 % CaCO_3): 6 weeks old: showing *a*, epiphyseal cartilage; *b*, well developed band of hypertrophied cartilage cells; *c*, wide overgrown band of hypertrophied cartilage cells, arranged in parallel columns, separated by blood vessels: only traces of calcification present; *d*, spongiosa with numerous well calcified trabeculae having normal marrow in the interstices: absence of the osteoporotic changes seen in Fig. 5 and 6—rickets. Note in addition the absence of calcification in the ossification centre in epiphyseal cartilage *e*.

Fig. 8. Tibia; chicken, group AD (basal + 1 % cod-liver oil); placed at 5 weeks of age into group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil): kept for 1 week on this diet—total disappearance of osteoporosis; bone now normal as in Figs. 2, 3, 4 (lettering as in Fig. 4).

Fig. 9. Tibia; chicken, group B (basal + 1.2 % CaCO_3); placed at 5 weeks of age into group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil): kept for 1 week on this diet—total disappearance of osteoporosis; bone now normal as in Figs. 2, 3, 4 (lettering as in Fig. 2).

Fig. 10. Tibia; chicken, group C (basal + 6 % CaCO_3); placed at 5 weeks of age into group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil): kept for 1 week on this diet—total disappearance of rickets; bone now normal as in Figs. 2, 3, 4 (lettering as in Fig. 4).

Fig. 11. Tibia; chicken, group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil); placed at 6 weeks of age in group B (basal + 1.2 % CaCO_3): kept for 3 weeks on this diet: osteoporosis of marked degree now present, modified slightly from the appearance in Fig. 5 by previous development and persistence of the zone of hypertrophied cartilage: *a*, epiphyseal cartilage replaced by bone consisting of a few slender trabeculae in a background of loose fibrous tissue; *b*, zone of hypertrophic cartilage cells; *c*, subepiphyseal zone with practically no trabeculae and consisting chiefly of a loose fibrous tissue; *d*, zone with few but rather large trabeculae.

Fig. 12. Tibia; chicken, group BD (basal + 1.2 % CaCO_3 + 1 % cod-liver oil); placed at 6 weeks of age in group C (basal + 6 % CaCO_3): kept for 3 weeks on this diet: shows marked rickets as in Fig. 7: note that there is no removal here of the calcified trabeculae of the normal bone but on the other hand marked proliferation of cartilaginous trabeculae in columnar arrangement from the zone of hypertrophied cartilage cells. These trabeculae remain practically uncalcified. Note also the lack of calcification in the cartilaginous trabeculae in the centre of ossification of the epiphyseal cartilage (lettering as in Fig. 7).

(The osteoporotic changes present at 6 weeks in groups AD and B as also the rachitic ones in group C were present also in specimens from these groups examined at 2, 3, 4 and 5 weeks of age.)

Addendum (August 26th, 1934). In a previous paper [McGowan, 1933, 3] it was suggested, in view of the remedial action of vitamin D in the disease, that osteomalacia was, in addition to being an acalcicosis, an aphosphorosis. As, however, vitamin D has been shown in the present experiments to have a marked influence on Ca absorption and metabolism; as P is abundant and Ca deficient in the cereal diets which give rise to osteomalacia; and as the addition of Ca is the most important factor in the cure of the condition, these circumstances, together with the histological appearances, render it more probable that osteomalacia is a pure acalcicosis.