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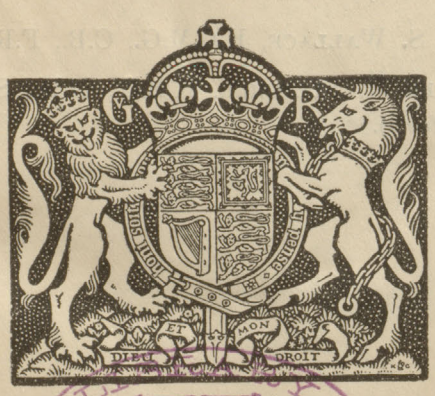
EXPERIMENTAL RICKETS

The Effect of Cereals and their Interaction with other Factors of Diet and Environment in producing Rickets

BY

EDWARD MELLANBY



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AL RICKETS

AND THEIR INTERACTION
OF DIET AND ENVIRON-
ICKETS.

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I. INTRODUCTION.

Investigations carried out during recent years on the subject of diet have not only brought to light the importance of quality of the food but have also emphasized the necessity of balance among some of the essential constituents. This need of balance of foodstuffs depends upon the interaction of different dietetic factors, which is of such a nature that alteration in the amount of one often necessitates change in another before normal development and function can result. The present publication deals with one aspect of this problem, namely, the interaction of food factors on bone calcification. Probably in no physiological activity so far studied do action and reaction of dietetic elements stand out more prominently than in the growth and hardening of bone.

It might be thought that a physiological process whose outcome was the deposition of calcium phosphate in growing bone would be relatively simple, and would be influenced to a large extent, or even solely, by the amounts of calcium and phosphorus in the diet. In previous publications [1 *a*, *b*, *c*, *d*], however, I have shown that this is not the case, but that, among other dietetic factors which influence the process, a calcifying vitamin, the anti-rachitic factor, plays a prominent part, and this has been amply confirmed by others. (Korenchevsky [23 *a*], McCollum, Simmonds, Shipley and Park [4 *c*.]) Another important food constituent influencing bone calcification, but in an opposite sense to the anti-rachitic vitamin, is found in cereal. (E. Mellanby, [1 *d*, *e*, *f*, *g*.])

Cereals have long formed the major part of the diet of man. From a physiological standpoint they have been considered almost entirely as sources of energy, of carbohydrate and of protein, and on this basis their dietetic value has been appraised. It is true that the presence of vitamin B in natural cereals, and its loss in the manufacture of many products, is an important fact to be reckoned with in any consideration of the relative nutritional value of different preparations of a cereal. For instance, the relative dietetic value of polished and unpolished rice centres round the vitamin B content of these foods. These observations have not, however, led to the belief that there are important differences in food value among the natural cereals themselves, except such as can be explained in terms of their known physiological constituents. The experimental work described here demonstrates that various common cereals have different effects on the growing animal, and that these differences cannot yet be explained on the basis of their known constituents.

It has long been recognized that rickets often develops in children whose diets contain much carbohydrate, and many clinicians, among whom may be mentioned Cheadle [2], have taught that carbohydrate and carbohydrate-containing foods are important aetiological factors in this disease. Sometimes the carbohydrate of cereal in the form of starch was blamed, while at other times the simpler polysaccharides

found in malted foods or in sweetened condensed milk were regarded as the offending dietetic substances. Little, if any, attempt seems to have been made either to place the suggestion on a definite scientific basis or to determine the relative importance of the different forms of carbohydrate or of carbohydrate-containing foods as causative agencies in rickets.

In earlier publications on experimental rickets in puppies (E. Mellanby, [1 *c*, *d*]) I showed that, under certain dietetic conditions, the intensity of the disease which developed was related to the amount of bread eaten. On diets the principal defect of which was a deficiency of anti-rachitic vitamin the rickets was most strongly developed in those animals which ate the most bread when all other factors of diet and environment were kept constant. The animals which ate most bread put on most weight, and, within limits, had a more rapid growth of bone. It appeared that the greater rate of bony growth resulting from increased bread consumption necessitated a greater intensity of calcification to keep pace with the development. If there was no corresponding increase of those factors concerned with calcification, more severe rickets would develop. As an explanation of the experimental results I accepted the view widely held that the starch in the bread was responsible for the rickets-producing action. I thought, however, that this view should be tested, and therefore attempted to feed puppies on diets deficient in anti-rachitic vitamin and whose carbohydrate content alone varied. Starch, cane sugar, and glucose were added as extra carbohydrate. Many of these experiments failed because of the difficulty of inducing puppies to eat quantitatively diets containing much pure carbohydrate, more especially as experiments of this nature must continue over three or four months before a satisfactory outcome can be attained. In one fairly satisfactory series of three puppies, where the experiment was carried on for an adequate period, the influence of the extra glucose ingested was not great, so far as defect in endochondral ossification of the bone was concerned, but became more obvious when comparison of the calcium present in the bones of the respective animals was made. For instance, the femur shaft of the puppy on the control diet without extra carbohydrate contained 14 per cent. of calcium oxide, while that of the puppy which had eaten an extra 50–90 gms. of glucose daily only contained 9·6 per cent. of this substance. That is to say, the more carbohydrate eaten at a time when the diet was deficient in anti-rachitic vitamin, all other factors of the diet and environment being kept constant, had resulted in greater bone defect. But while this was the case, I did not feel satisfied that the rickets-producing effect of bread in the earlier experiments was solely due to its carbohydrate moiety, and suggested that 'bread may contain other offending constituents' [1 *d*].

In the same publication it was suggested, although the point had not been tested by experiment, that other cereals would be expected to behave in a similar way to bread, and that increasing the amount of oatmeal and rice in the diet would, under the conditions described, also intensify the rachitic condition produced. A further suggestion was made that cereals would prove to differ in their

rickets-producing effects; that, for instance, oatmeal would be expected to have a less intense rickets-producing action than white wheaten flour, and that unpolished rice would not be as potent as polished rice. These suggestions were based on the view that a cereal like oatmeal, which contains much more calcium and phosphorus than wheaten flour, would either assist in the deposition of these elements in growing bone or, at least, would have a slighter interfering action. From this standpoint the suggestion not only seemed reasonable, but it might be further claimed to have the general support of those interested in dietetics. However, the results of experiments described below will show the danger of any surmise on the subject of dietetics, when the point has not been tested experimentally.

I have already described many of the experimental results detailed in this publication when reading papers to or giving lectures before different societies, among which may be mentioned the Oliver-Sharpey lectures of the Royal College of Physicians in 1922 [1 *e*] and the discussion on the aetiology of rickets at the Glasgow meeting of the British Medical Association in the same year [1 *f*]. Since that time I have endeavoured to get some insight into the sequence of events and more especially to find the substance or substances in cereals which, under some conditions, interfere with the calcification of bone and the proper functioning of other tissues. This problem has proved more difficult than I expected, for its solution does not appear to depend, as seemed at first probable, upon some well-recognized dietetic factor or factors. The elimination of each known factor in turn is a slow process, but it is only when this has been done that fresh ground can be broken. Although the investigation is still incomplete, I have decided to publish the facts as I have so far observed them, partly because of their practical importance in dietetics, and also because they put into better perspective one aspect of recent work on rickets, more particularly emphasized by American investigators, viz. the question as to the importance of the phosphorus and calcium elements of the diet in the aetiology of the disease.

Further, as I have previously urged, they give emphasis to the importance of balance in dietary constituents.

I shall first give an account of experiments which demonstrate the relative rickets-producing[1] effect of different cereals, and then describe some attempts made to discover the cause of these differences. Evidence will also be given which shows that the rickets-producing effect of cereals can be modified to varying extents not only by other constituents of the diet but also by radiations of the ultra-violet lamp applied either to the skin or some foodstuffs. In some instances the antagonizing influences can completely overcome the effect of cereals, whereas in other cases the antagonism is not so complete. It will be seen that there is a constant battle going on among dietetic elements as regards the calcification of bone, some substances stimulating and others preventing the process, and the ultimate structure of the bone is the outcome of the conflict.

[1] The term 'rickets-producing' is used throughout this publication in a general sense and does not necessarily imply the presence of an active and specific agent.

The present investigations seem to show that it is useless to attempt to explain problems connected with calcium and phosphorus metabolism in terms of mineral salts alone. More light will only be thrown on the subject when a clearer view of all the forces engaged and of their mode of action is obtained.

II. EXPERIMENTAL METHODS.

I have previously described the feeding technique of this experimental work on puppies. The majority of the experiments recorded in this publication was carried out at the Field Laboratory, Sheffield University. The conditions were more uniform than those at Cambridge, where much of the earlier work was performed.

The basal diets used as a rule consisted of separated milk, usually in the form of a powder, meat from which all visible fat was removed, cereal, orange juice, yeast, sodium chloride, and a fat. The fat used was generally olive oil when the effect of cereals was being tested. In most of the experiments described below the cereal of the diet has been the special object of investigation, and has therefore been the chief variable. These diets are not composed of purified foodstuffs, each of which represents a dietetic unit. It would be wellnigh impossible to carry out many feeding experiments on dogs along lines identical with those used in the rat experimental work. Even in the rat work the use of complex substances, such as yeast and cereal, in many of the synthetic diets, lays these investigations open to a criticism similar to that sometimes urged against the diets used in the experiments on dogs. The diets of the experimental puppies have the merit of being eaten quantitatively with relish, so that each member of a litter eats the desired *amount* of food. In this respect the feeding experiments on rats usually fail. In addition, puppies grow well on these diets, at least in the early stages, and the development of rachitic symptoms, both as regards rate of onset and intensity, can be fairly well controlled by alterations in their composition.

These qualities, and the additional ones of relative cheapness and ease of preparation, far outweigh the defect of being more obscure in their composition than are some of the diets used in the rat work. Further, no one can investigate the action of food without feeling that there are other dietetic factors still undiscovered which play an active part in the general nutrition of animals. In fact, this investigation dealing with the effect of cereals on rickets strongly indicates the presence of some hitherto unknown dietetic factor. It is probable that such unknown substances in diet can best be brought to light by working on foodstuffs as eaten by man.

In this publication attention has been fixed on the bones, but of course it is needless to add that, as in the human disease, many other organs are influenced as the result of the dietetic changes. Some of these dealing with muscle have already been described to the Physiological Society by Clifford, Surie, and myself, and will be published separately.

The methods used for determining the bone changes included, as

before: during life, (1) general appearance and activity, (2) radiographic examination; and after death, (3) histological examination and (4) the estimation of the calcium content of the bones. It is unnecessary to repeat the details of the methods and the difficulties encountered, as these have been dealt with in a previous publication, but one or two points as regards the correlation of the different results might well be mentioned. When an animal continues to eat its diet completely to the end of the experiment, the various records obtained by radiographic, microscopic, and chemical examination, as well as the general behaviour and appearance of the animal, will indicate the complete result, and each record will be in harmony with the other. But one or more puppies of a series may develop severe rickets in the course of the experimental period, and as a result begin to leave part of their food. When this happens, since each animal compares with the others of the series, an attempt is made to keep the animals comparable by reducing the diet given to all the members of the series. Often it becomes impracticable to reduce the general diet sufficiently, and, at this stage, either the experiment is terminated or, if continued, those animals still refusing to eat their rations are left behind. When the reduction in diet takes place, as I have previously pointed out, cessation in the progress of the disease and even some recovery in the rachitic changes may follow, so that at the end of the experiment radiographic and histological appearance and chemical composition of the bones suggest a condition better than was present several weeks before, as indicated by X-ray examination and general behaviour. Other dogs in the same series may have continued to eat their diets and, if these are rickets-producing, will have got steadily worse to the end. Thus the end result will not always necessarily record the real state of affairs, because in the worst cases the disease may have improved, and in the slighter cases got worse during the later weeks. This difficulty, depending upon the rapid development of very severe rickets and subsequent recovery when the diet is not completely eaten, concerns more particularly the experiments in which oatmeal is the cereal of a diet deficient in anti-rachitic vitamin. Reference to the weight curves will show that, again and again, the animals whose diet contains oatmeal as the cereal begin to lose weight after a period. This loss of weight coincides with the development of severe rickets (see Figs. 2 and 3). The rapidity and severity of the disease which develops under these conditions is generally great, and it is in the case of these animals especially that the results have to be interpreted with discrimination. Thus, in order to overcome some part of the difficulty, I have tried in most cases to place side by side the radiographs of animals of a series taken before the healing process has commenced in any of the animals, i. e. before they have become ill and refused to eat their food completely. The radiograph at this early stage may show very bad rickets, whereas the structure and the calcium content of the bones at the death of the same animal may not suggest such severe rickets because of the healing process that may have set in. In a few cases the earlier radiographs have not been reproduced, and the later ones show healing changes, and thus do not indicate how

severe the rachitic condition has been when at its maximum. I have marked on the weight curves the time when the radiographs used as illustrations were taken. The wrist joints of all experimental animals are usually radiographed at intervals of a few weeks. This mode of examination has the merit of affording an accurate record of the progress of the disease.

It is often difficult to realize the profound general changes that are found in animals in association with what at first sight may seem to be but slightly abnormal radiographic appearance. I have therefore included in the illustrations a few photographs of dogs, which allow the comparison between the general appearance of the animal and its radiograph to be made.

The interpretation of the calcium content of the bones also presents difficulties at times. When comparison is being made between the effect of diets containing abundant anti-rachitic vitamin with those containing little, the difference between the calcium in the bones in the two cases is so great as to overshadow minor influences. When, however, the action of different cereals is being compared, the diets are also deficient in the calcifying vitamin, so that the calcium content of the bones is generally low, and differences are relatively smaller. In such circumstances extraneous influences become more prominent, so that, for instance, a period of ill health involving loss of appetite, before the end of the experimental period, may alter the percentage of calcium in the bone so as to put it apparently out of harmony with the other manifestations of rickets. This condition becomes of particular import in the figures representing the percentage of calcium oxide in the dried bone. Loss of appetite and loss of weight in puppies are often accompanied, as I have previously pointed out, by disappearance of fat from the bone marrow, and its place is taken by the fluids of the body. On drying the bone, much of its weight is lost owing to evaporation of water, whereas in the case of the bone of the healthier animal the fat remains after drying, and the difference between the weights of the dry and fresh bone is less. Thus, in the case of an animal which has stopped eating its food the percentage of calcium oxide in terms of the weight of the fresh bone may be low, but of the dried bone comparatively high. Ill health and loss of appetite in these experiments are usually the outcome of potent rickets-producing diets, so that a somewhat high percentage of calcium oxide in terms of dried bone is disconcerting, but, in view of this explanation, of no great significance. In order to assist in the interpretation of the calcium results I have given the total amount of calcium oxide in the femur shaft as well as its percentage weight. It will be noticed that this figure is low in bad cases of rickets and higher in more normal animals.

The fact is that when big differences, produced by a particular condition, are being studied, any of the methods used will provide definite evidence of the action. There is never, for instance, any difficulty in deciding that oatmeal, under the circumstances described, has a much greater rickets-producing influence than white flour. When smaller differences have to be observed, all the results must be marshalled and examined, and a decision come to

after judgement of the whole. The general condition and appearance of the animal, its activity and ability to run, are also of great importance to the observer, but these indications are more difficult to record and describe with accuracy.

In each series of experiments the puppies were of the same litter.

III. INFLUENCE OF CEREALS ON RICKETS.

(A) The Relation of the Intensity of the Disease to the Amount of Cereal eaten.

The following experiments, typical of a number carried out, show that just as increasing the bread of a diet deficient in anti-rachitic vitamin increases the intensity of rickets in puppies (E. Mellanby 1. *d*), so also an increase in the amount of another cereal, in this case oatmeal, intensifies the bone defects.

Influence of increasing Oatmeal in a Diet deficient in Anti-rachitic Vitamin.

Age at beginning of experiment: 7 weeks.

General diet consisted of separated milk powder 15 gms., meat 10 gms., linseed oil 10 c.cms., orange juice 3 c.cms, yeast 5 gms., sodium chloride 1 to 4 gms.

In addition to these substances:

502 received 60 gms. oatmeal daily in the form of porridge.

509 „ 120 gms. „ „ „ „ „

These animals lived out of doors during the day time throughout the experimental period.

Table 1.

No. of Expt.	*Diet. Variable.*	*Duration of Expt. Weeks.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial. gms.	*Final. gms.*	*Max. gms.*	*Percentage.*		*Amount. gms.*	
						Dry.	*Fresh.*		
502	Up to 60 gms. Oatmeal	18	1080	3280	3280	17·3	11·1	0·57	Rickets
509	Up to 120 gms. Oatmeal	18	1255	2970	3450 after 13 weeks	12·5	7·6	0·35	Bad rickets

The radiographs taken after 9 weeks of the diets (Figs. 1 and 2), the calcium contents of the bones, and their histological structure make it evident that 509, the puppy which had eaten the larger amount of oatmeal, developed the worse rickets. It will be further noted that (Fig. 3), although 509 put on weight more rapidly than 502, owing to the greater amount of oatmeal eaten, this reached a maximum after 13 weeks of the experimental period, and thereafter the animal lost weight while 502 continued to grow until the end of the experiment. As explained above, this is a common experience in feeding experiments when oatmeal is the cereal eaten, especially if in large quantities, and the diet is also deficient in anti-

rachitic vitamin. Under these circumstances the animals develop rickets very rapidly and intensely, and thereafter refuse to eat their diet quantitatively. As I have previously shown, a diminution in amount of food eaten often results in curative changes, so that at the end of the experimental period the differences in intensity of the disease may not be so prominent as at an early stage.

Further examples showing the effect of increasing the cereal element in the diet, other factors being kept constant, will be seen in Experiments 712 and 713, 714 and 717 (see Table 17 and Figs. 64, 65, 74, and 75, also 66, 69, 76, and 79).

It may be concluded that, when the diet is deficient in anti-rachitic vitamin, increasing the amount of cereal eaten, either oatmeal or white flour, intensifies the severity and increases the rate of onset of rickets.

(B) The Variation in the Rickets-Producing Effect of Different Cereals.

Experiments will now be described which demonstrate the relative rickets-producing effect of equal amounts of different cereals.

Comparison between White Flour, Whole Meal Flour, Oatmeal, and Unpolished Rice.

Experiments 418, 419, 421, *and* 422.

Age at beginning of experiment: 8 weeks.

The general daily diet eaten by all consisted of separated milk powder 20 gms., meat 10 gms., olive oil, 10 c.cms, lemon juice 5 c.cms., yeast 5 gms., and sodium chloride 1 to 3 gms.

In addition 418 received 30–150 gms. white flour
„ 419 „ „ unpolished rice
„ 421 „ „ oatmeal
„ 422 „ „ whole meal flour.

The cereals were cooked in separate compartments of the same steamer.

Table 2.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial.	*Final.*	*Max.*	*Percentage.*		*Amount.*	
		Weeks.	*gms.*	*gms.*	*gms.*	*Dry.*	*Fresh.*	*gms.*	
418	White flour	14½	1450	3580	3580	22·2	14·6	0·74	Some rickets
419	Unpolished rice	12	1410	3500	3500	21·7	12·5	0·71	Rickets
421	Oatmeal	14½	1680	4430	4430	13·3	7·35	0·46	Very bad rickets
422	Whole meal flour	14½	1320	4250	4250	19·0	10·2	0·60	Bad rickets

The radiographs of these animals, taken after 14 weeks of the diet (except 419, 12 weeks), can be seen in Figs. 4, 5, 6, and 7. 421 (oatmeal) has developed rickets most severely. Of the others

422 (whole meal flour) is worse than 419 (rice) and this is a little worse than 418 (white flour).

The histological appearance of the bones shows that oatmeal has produced much the worst rickets in this series; then come whole meal flour, unpolished rice, and white flour, which has produced least rickets.

The calcium content of the bones is also in agreement, the order being oatmeal (worst), whole meal flour, rice, and white flour. The general appearance and activity of these animals lead to the same conclusion. 421 (oatmeal) was the most rachitic in appearance and least active. 418 (white flour) was the most normal animal of the series and quite active. 419 (unpolished rice) was not so active as 418 (white flour) but was better than 422 (whole-meal flour), whose movements were limited after 10 weeks of the diet.

The rice experiment (419) was terminated two weeks before the others because the puppy was suspected of having developed distemper. Up to within 2 or 3 days of its death this animal was quite well and continued to increase in weight to the end.

One other point in this series must be mentioned, namely, the fact that the difference in the degree of rickets between 422 (whole meal flour) and 418 (white flour) was greater than was found in other similar experiments. This might possibly be accounted for by the type of whole meal flour used in this experiment, which was carried out in 1921 at Cambridge. It will be noticed, also, from the weight curves of this series (Fig. 8) that 422 (whole meal flour) increased in weight more rapidly than 418 (white flour), although the diets were closely comparable. This greater increase in weight would, no doubt, accentuate the difference in the intensity of rickets which developed in these animals.

The rickets-producing order in these experiments came out:

(1) Oatmeal (much the worst).
(2) Whole meal flour.
(3) Rice.
(4) White flour.

Whereas oatmeal was much worse than whole meal there was not a great difference between the other three cereals.

Comparison between White Wheaten Flour, Whole Meal Flour, Oatmeal, Barley, Polished Rice and 'Commercial Germ' and Polished Rice.

Experiments 460, 461, 462, 463, 464, *and* 465.

Age of puppy at beginning of experiment: 6 weeks.

The general daily diet eaten by all included separated milk powder 15 to 20 gms., olive oil 10 c.cms., meat 20 gms., yeast 10 gms., sodium chloride 1 to 4 gms., orange juice 5 c.cms.

In addition 460 received 30–160 gms. white flour
„ 461 „ „ whole meal flour
„ 462 „ „ oatmeal
„ 463 „ „ barley
„ 464 „ „ polished rice and 20 per cent 'commercial wheat germ'
„ 465 „ „ polished rice.

The amount of cereal given to the puppies throughout the experimental period was kept equal except in the case of 464 (rice + commercial germ). In this case the amount of rice eaten was the same as that eaten by 465 but the 'commercial wheat' germ was additional, so that this animal (464) was actually given more cereal than any of the other puppies, and it is therefore not strictly comparable with the others. 'Commercial wheat germ' contains bran which, unfortunately, varied in amount in different specimens supplied by the millers. In the case of 463 (barley) there was also a drawback which interfered with the strict interpretation of the result. In this case the whole barley grain, including husk, was crushed, so that there was more indigestible matter in this diet than in that of the others. This animal (463), therefore, got less digestible cereal than the others and consequently put on a little less weight. (See Fig. 15.) In all cases the cereals were cooked by steaming for the same length of time in the same apparatus.

TABLE 3.

No. of Expt.	*Diet. Variable.*	*Duration of Expt. Weeks.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial. gms.	*Final. gms.*	*Max. gms.*	*Percentage.*		*Amount. gms.*	
						Dry.	*Fresh.*		
460	White flour	18	1400	4050	4050	19·0	10·9	0·55	Slight rickets
461	Whole meal flour	18	1420	4020	4020	18·0	10·2	0.62	Slight rickets
462	Oatmeal	18	1410	2920	3140 after 17 weeks	20·7	11·1	0·39	Very bad rickets
463	Barley	18	1290	3300	3300	20·4	12·1	0·45	Very bad rickets
464	Polished rice and wheat germ	18	1600	3740	3870	16·1	9·3	0·57	Very bad rickets
465	Polished rice	18	1450	3590	3690	21·4	13·2	0·74	Rickets

The radiographs of these animals taken after 12½ weeks of the experimental period are to be seen in Figs. 9, 10, 11, 12, 13, and 14. The order of severity of the bone changes is as follows:—462 (oatmeal), Fig. 11, and 464 (rice and wheat germ), Fig. 13, worst, then come 463 (barley), Fig. 12, 465 (polished rice), Fig. 14, 461 (whole meal), Fig. 10, and 460 (white flour), Fig. 9, with the least rickets. At this stage of the experiment there is but little difference between 463 (barley), 465 (polished rice), 461 (whole meal), and 460 (white flour). According to later radiographs (not shown) taken after 18 weeks of the diets, 463 (barley) developed more severe rickets relatively than 465 (polished rice). 461 (whole meal flour) was similar to 460 (white flour) throughout the experiment, and both only developed slight rickets.

The histological appearance of the bones showed that 462 (oatmeal), 465 (polished rice and germ), and 463 (barley) had developed very bad rickets by the end of the experimental period.

465 (polished rice) was more rachitic than 461 (whole meal flour) and 460 (white flour). The last two had only slight rickets of similar intensity.

But little can be inferred from the percentage calcium content of the bones, partly because the calcification of all the bones was poor, since the diets were deficient in anti-rachitic vitamin, and partly because, in the case of 462 (oatmeal), failure to eat its food completely each day in the later part of the experiment brought about a diminished rate of increasing weight, so that curative changes actually began in this animal, as can be seen in the last radiographs taken. The weight curve (Fig. 15) shows that in the last six weeks of the experiment the weight of 462 (oatmeal) was almost stationary. It will be noticed also that up to the time the radiographs (Figs. 9–14) were taken, i.e. $12\frac{1}{2}$ weeks, these animals gained in weight to the same extent except 463 (barley) which advanced a little more slowly for the reason given above and 464 (rice + germ), which put on weight rather more rapidly than the others because the wheat germ of its diet was additional to the amount of cereal eaten by the other dogs. With the development of rickets at a later stage this animal (464) ate less food and its weight increased at a slightly slower rate.

Photographs of some of the puppies of this series after 15 weeks of diet are also reproduced (Figs. 16, 17, 18 and 19). The rachitic appearance of 462 (oatmeal), Fig. 17, and of 464 (rice and wheat germ), Fig. 18, is in striking contrast to the more normal appearance of 460 (white flour), Fig. 16, and 465 (polished rice), Fig. 19. 461 (whole meal flour) was also almost normal in appearance.

The conclusion to be drawn from this series of experiments is that the rickets-producing effect of the different cereals is in the following order: oatmeal (most), barley, polished rice, whole meal, and white flour (least).

The addition of commercial wheat germ to rice certainly intensified the rachitic condition, but the increase in cereal intake would in itself explain some increase in the rickets. The disease in 464 was, however, so much more intense than in 465 that it appeared probable that the germ had exerted a specific rickets-producing effect of its own. Experiments made to test this point will be described later.

Comparison between Rice, Oatmeal, White Flour, and Wheat Germ.

Age at beginning of experiment: $9\frac{1}{2}$ weeks.

The general daily diet eaten by all included separated milk powder 15 gms., meat 20 gms., olive oil 10 c.cms., yeast 5 gms., orange juice 3 c.cms., sodium chloride 2 gms.

In addition 589 received 50–120 gms. rice.
„ 590 „ „ oatmeal
„ 591 „ „ white flour
„ 592 received 40–100 gms. white flour and 20 per cent. wheat germ.

In this series, unlike the previous series of experiments (Table 3) and others not described, where the commercial wheat germ eaten

was additional to its cereal ration of rice, 592 received wheat germ as a substitute for 20 per cent. of the white flour of its diet. Thus when 591 ate 100 gms. of white flour, 592 got 80 gms. white flour and 20 gms. germ. These animals, therefore, received the same quantity of cereal daily. As in previously described experiments, the cereals were cooked under the same conditions and for the same period. These animals were older ($9\frac{1}{2}$ weeks) at the beginning of the experiment than those of most other series. They were born in the laboratory and fed on an excellent diet prior to the experimental period.

TABLE 4.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial.	*Final.*	*Max.*	*Percentage.*		*Amount.*	
		Weeks.	*gms.*	*gms.*	*gms.*	*Dry.*	*Fresh.*	*gms.*	
589	Rice	17	2020	5100	5100	21·6	14·1	0·89	Bad rickets
590	Oatmeal	17	1550	4070	4070	19·3	11·3	0·61	Very bad rickets
591	White flour	17	1570	4920	4920	21·6	14·2	0·99	Rickets
592	White flour and wheat germ	17	2010	5100	5100	22·7	12·9	0·80	Bad rickets, not so bad as 590

The radiographs of these animals (Figs. 20, 21, 22, and 23), taken after 17 weeks of the diet, show that 590 (oatmeal), Fig. 21, developed the most severe rickets. There was no great difference in the intensity of the rachitic changes in the other animals of this series, but 592 (white flour and germ), Fig. 23, was slightly worse than 591 (white flour), Fig. 22.

The histological and calcium results are in general agreement with the radiographic evidence. According to the microscopic structure 590 (oatmeal) has much the worst rickets, while 591 (white flour) has the least. 589 (rice) and 592 (white flour + wheat germ) have bad rickets, worse than 591 (white flour) but less severe than 590 (oatmeal). Of the two 592 (white flour + wheat germ) is rather worse than 589 (rice).

It will be seen from the calcium content of the femur shafts that the degree of bone-calcification in the dogs of this series is in the order, 590 (oatmeal) worst, then 592 (white flour + germ), 589 (rice) and 591 (white flour) best.

As regards general appearance and speed in running, the order of rachitic severity was again 590 (oatmeal) worst, then 592 (white flour + germ), 589 (rice) and 591 (white flour) best.

The weight curve of this series of animals is shown in Fig. 24. It will be seen that except for 590 (oatmeal), which only put on a small amount of weight during the last 60 days, the rate of increase in weight of these animals was remarkably constant. The lag in growth of 590 (oatmeal) was again due to inability to finish off

its daily ration owing to the development of severe rickets. As pointed out in previous experiments this is a common result when oatmeal is the cereal eaten. In this series of experiments commercial wheat germ, even when replacing white flour so that the total cereal intake was constant, has made the rachitic condition worse, although the relative increase in intensity of rickets is not so great as was found in puppy 464 of a previous series (Table 3) where the germ was additional to the white flour.

The order of rickets-producing power of the cereals tested in this experiment is:

(1) Oatmeal (worst).
(2) White flour + commercial wheat germ.
(3) Rice.
(4) White flour (best).

The Rickets-producing Effect of Wheat Germ.

In the following experiments commercial wheat germ was again substituted for part of the white flour, so that the total cereal eaten by the two animals was the same.

Age at beginning of experiment: 7 weeks.

General daily diet included separated milk powder 20 gms., meat 10–20 gms., yeast 3–5 gms., orange juice 3 c.cms., sodium chloride 1–3 gms., olive oil 3–10 c.cms.

In addition 824 received 30–90 gms. white flour
and 825 „ „ a mixture of white flour and commercial wheat germ, in the proportion of 60 per cent. white flour and 40 per cent. germ.

The cereals in each case were equally cooked.

TABLE 5.

No. of Expt.	*Diet. Variatle.*	*Duration of Expt. Weeks.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial. gms.	*Final. gms.*	*Max. gms.*	*Percentage. Dry.*	*Percentage. Fresh.*	*Amount. gms.*	
824	Control	17½	1070	3575	3575	18·1	10.4	0.387	Bad rickets
825	Wheat germ	16½	1060	3075	3285 after 10½ weeks	17·1	6·9	0.317	Bad rickets, worse than 824

The radiographs of Exps. 824 and 825 (Figs. 25 and 26), taken after 11 weeks of the experimental diets, show that both animals developed rickets, but that 825 (wheat germ) was worse than 824 (control).

Although both developed bad rickets according to the histological appearance of the bones, 825 was the worse, for there was hardly any attempt at calcification at the costo-chondral junctions, whereas in 824 some calcification at this place was evident.

It is also obvious from the calcium oxide in the femur shafts of the two animals that the calcification of the bones in 825 (wheat germ) was more defective than in 824 (white flour).

Thus the substitution of wheat germ for white flour to the extent of 40 per cent. made the rachitic condition worse.

Further examples showing the effect of wheat germ when substituted for white flour can be seen in Exps. 806 and 807 (Table 21, see also Figs. 89 and 90).

Comparative Effect of Ground Oats, Groats, Oatmeal, Rye, and White Flour.

In the experiments so far described one constant factor has stood out prominently, namely, that the rachitic changes produced by oatmeal have been, under the experimental conditions, more intense than those found in the case of animals eating other cereals which have been tested. It seemed possible that some of this action might be due to the fact that it was a manufactured product and that the natural oats might not have the same effect. The following experiments were therefore carried out to see whether there was any difference between corresponding quantities of oatmeal, home-ground oats, and groats in their rickets-producing effect.

Age at beginning of experiment: 7 weeks.

The general daily diet eaten by all animals in the series consisted of separated milk powder 20 gms., meat 10-20 gms., olive oil 5–10 c.cms. (except 742), yeast 5 gms., orange juice 3 c.cms., sodium chloride 1–3 gms.

In addition 743 received 40–120 gms. white flour
„ 744 „ „ groats
„ 745 „ „ rye
„ 746 „ „ oatmeal
„ 747 received 50–145 gms. whole ground oats.

742, another puppy of this series, referred to elsewhere (see p. 21) received the same diet as 746 (oatmeal), except that 3 of the 10 c.cms. of olive oil eaten by 746 were replaced by cod-liver oil.

The oats eaten by 747 were finely ground in the laboratory. Since the husk and all parts of the grain were included, that is to say much indigestible matter, more oats were allowed this animal than cereals to the other puppies. From the rate of increase in weight it appeared that 72 gms. ground whole oats were approximately equivalent to 60 gms. oatmeal and other cereals, so that 747 was allowed one-fifth more cereal than the other animals of the series.

Groats which consist of oats without the husk were also ground up finely before cooking and were given in the same amount as the other cereals.

As in previously described experiments, all the cereals were equally cooked in separate receptacles of the same apparatus.

It will be seen, from the radiographs (Figs. 28, 29, 30, 31, and 32), taken after 9 weeks of the diets, that the intensity of the rachitic condition was closely similar in 746 (oatmeal), Fig. 31; 747 (whole oats), Fig. 32; and 744 (groats), Fig. 29; also that these animals

developed the disease more severely than 745 (rye), Fig. 30, and 743 (white flour), Fig. 28.

Although the calcium in the bones of these animals is small in all cases, it is distinctly lower in 746 (oatmeal) and 747 (whole oats) than in 743 (white flour) and 745 (rye). The bones of 744 (groats) were not examined by this method because the animal, after developing most severe rickets, was cured by change of diet and used for other purposes.

TABLE 6.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.* *Weeks.*	*Weight.* *Initial.* *gms.*	*Final.* *gms.*	*Max.* *gms.*	*CaO in Femur Shaft.* *Percentage.* *Dry.*	*Fresh.*	*Amount.* *gms.*	*Histology.*
742	Oatmeal and 3 c.cms. c.-l.o. substituted for 3 c.cms. olive oil	Unfinished	—	—	—	—	—	—	—
743	White flour	16½	1570	4265	4300 after 14 weeks	20·5	10·9	0.64	Rickets
744	Groats	Unfinished	1515	—	—	—	—	—	—
745	Rye	16½	1455	2600	3600 after 13 weeks	26·6	10·6	0·57	Rickets
746	Oatmeal	15	1490	3960	4120 after 14 weeks	20·0	7·9	0·47	Very bad rickets
747	Whole oats	14	1520	3665	3665	20·9	8·2	0·47	Very bad rickets

The histological results are in agreement with the calcium contents of the bones, and indicate that 746 (oatmeal) and 747 (whole oats) had developed the rachitic condition to equal degrees of severity, and that both were much worse than 743 (white flour) and 745 (rye).

The weight curves of these animals are shown in Fig. 33.

In this experiment it was found that the rickets-producing effect of rye was somewhat of the order of white flour. Several experiments have been made using rye as the cereal, but in no case has the outcome been entirely satisfactory. Even in Exp. 745 (rye) the puppy did not eat its diet completely in the early stages and again during the last month of the feeding period (Fig. 33). Whether the unsatisfactory outcome of experiments with rye is due to misfortune or depends upon some toxic action of rye when eaten under these experimental conditions is not known. As regards the other results of this series, it would appear that whole oats and groats are as strongly rickets-producing as oatmeal, and it can be deduced that the intensity of the action of oatmeal is not due to some change undergone in manufacture. In these experiments the whole oats, groats, and oatmeal proved more intensely rickets-producing than rye or white flour.

Summary.

Experiments on puppies have been described in which cereals were varied in diets deficient in anti-rachitic vitamin. It has been shown that equal weights of similarly cooked cereals have different rickets-producing effects on puppies. Of the substances tested, oatmeal, groats, or ground oats had by far the most intense action. White flour had the least action in this respect. Rice occupied an intermediate position and was somewhat worse than white flour. Barley appeared to be more rickets-producing than rice, but further work on this cereal is necessary. From other experiments not recorded above it would appear that maize is more rickets-producing than wheat, but is not so potent in this respect as oatmeal. Although the point was not examined closely, there seemed to be no great difference between the rickets-producing effects of polished and unpolished rice. The action of whole meal flour was not dissimilar from that of white flour. Commercial wheat germ, however, when forming 20 per cent. of the total cereal eaten, hastened the onset of rickets under the conditions of these experiments. It is said that whole meal flour contains only 1·5 per cent. germ (Osborne and Mendel [3]), so that the little difference in effect between white flour and whole meal flour in these experiments might be expected in spite of the action of commercial wheat germ above described. The intense rickets-producing effect of oatmeal does not seem to depend on the fact that it is a manufactured product, for crushed whole oats and groats were just as potent as oatmeal.

IV. THE INTERACTION OF CEREALS WITH OTHER ELEMENTS OF THE DIET.

So far experiments have been described which deal primarily with the effect of cereals on calcification, and evidence has been adduced which seems to show that there are one or more constituents of some cereals which interfere directly with calcification processes.

In earlier publications I have supplied evidence that a fat-soluble vitamin (anti-rachitic vitamin) is particularly concerned in promoting the calcification of bone [1. *a*, *b*, *c*, *d*]. I wish now to describe experiments made primarily to see how foodstuffs containing these different dietetic factors, the one class promoting and the other interfering with calcification, work in relation to one another and to other dietetic and environmental influences. While but little is known of the chemistry of these substances and even less as to how they act in the body in bringing about such profound changes, yet it seemed necessary to investigate the question of interaction among themselves and to see the degree to which each could modify the influence of the other. Only in this way could adequate appraisement be made of their relative importance. It is too early to suggest that this knowledge has been obtained, or even to deny that there are still important factors at work in relation to the problems of bone calcification, about which nothing is yet

known. However, in spite of realizing this difficulty, the practical importance of the problem would appear to justify the attempt to correlate the facts as we know them at present.

A. The Interaction of Cereals with Substances containing the Anti-Rachitic Vitamin.

I shall first show how the rickets-producing influence of diet, even when most potently active, as, for instance, when oatmeal is the only cereal eaten, can be completely antagonized by the anti-rachitic vitamin. In the following series of experiments the effect of different foodstuffs containing this vitamin will be described.

(i) *Cod-Liver Oil.*

Experiments 713 *and* 716.

Age at beginning of experiment: 6 weeks.

General daily diet: separated milk powder, 25–30 gms.; meat, 10–20 gms.; sodium chloride, 1–4 gms.; yeast, 3–5 gms.; orange juice, 3–5 c.cms.; oatmeal, 40–100 gms.

In addition, 713 received 10 c.cms. olive oil.
716 „ 10 „ cod-liver oil.

Table 7.

No. of Expt.	*Diet. Variable.*	*Duration of Expt. Weeks.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial. gms.	*Final. gms.*	*Max. gms.*	*Percentage.*		*Amount. gms.*	
						Dry.	*Fresh.*		
713	Olive oil, 10 c.cms.	22	2430	5590	5590 after 17 weeks	14·2	5·7	0·51	Very bad rickets
716	Cod-liver oil, 10 c.cms.	23	2270	6060	6060	23·1	12·1	1·53	Normal

It is clear from the above chemical and histological results, and from the radiographs (Figs. 65 and 68), that 10 c.cms. of cod-liver oil daily has completely antagonized the rickets-producing effect of the oatmeal eaten and allowed the development of a normal animal with well-calcified bones. It will also be noticed that, although the amount of calcium in the diets of these animals was the same, there is three times as much calcium oxide in the femur shaft of 716 (cod-liver oil) as in that of 713 (olive oil), i.e. 1·53 gm. as compared with 0·51 gm.

The weight curves of these animals (713 and 716) can be seen in Fig. 80. 713 (deficient vitamin) began to leave some of its food after ten weeks of the diet, owing to the development of severe rickets, and after this time did not gain much weight.

The extraordinary potency of cod-liver oil in antagonizing the effect of oatmeal on bone calcification can be better appreciated in the following experiments, where one animal (Expt. 742) received only 3 c.cm. per diem, and remained in good condition in spite of eating a fairly large quantity of oatmeal daily.

Experiments 742 *and* 746.

Age at beginning of experiment: 7 weeks.

General daily diet: separated milk powder 20 gms., meat 20 gms., oatmeal 40 to 120 gms., yeast 5 gms., orange juice 3 c.cms.

In addition, 742 received 3 c.cms. cod-liver and 7 c.cms. olive oil.

and 746 „ 10 c.cms. olive oil.

The only difference in the diet of these animals is that 3 c.cms. of olive oil is replaced by 3 c.cms. of cod-liver oil in the daily ration of 742. The effect of this small change was great, as can be seen in Figs. 27, 27 *a*, and 31 and 31 *a*, which are radiographic and photographic results respectively obtained with these puppies. In the case of 742 the calcium content of the bones and their histological structure was not determined, as the animal is still alive. In later experiments it was found that even 1 c.cm. of cod-liver oil daily was capable of antagonizing fairly effectively the influence of oatmeal.

(ii) *Milk, Separated Milk, and Butter.*

It was clearly necessary to see how the rickets-producing effect of cereals was influenced by milk and its products, and some experiments were carried out to this end.

To those who have studied the effects of milk and butter on different physiological processes, more especially in connexion with questions of growth and rickets, the results have often presented difficulties when viewed from the standpoint of the vitamin hypothesis. I pointed out in a previous publication [1. *d*] some of the difficulties presented by the results obtained in experiments designed to test the anti-rachitic action of butter. I found, among other things, that the anti-rachitic action of butter is small unless there is a corresponding amount of calcium salts in the diet to balance it. In this respect butter is different from cod-liver oil, because cod-liver oil exerts its calcium-retaining properties very potently, even when the calcium of the diet is very small (see Exp. 495 and 496, Table 16, p. 36). It may be that this difference between cod-liver oil and butter depends upon the fact that there is a much greater amount of the anti-rachitic vitamin in cod-liver oil than in butter, just as its growth-promoting properties are greater, as shown by rat-feeding experiments. It is interesting to note that, even as regards the power of these substances to promote growth, reproduction, and general well-being in rats, McCollum, Simmonds, Shipley, and Park [4] have found that butter is relatively impotent as compared with cod-liver oil unless the calcium of the diet is high. Thus these investigators found that a diet containing 3 per cent. butter and 0·5 per cent. calcium carbonate had the same physiological results on young rats as the same standard diet to which 2 per cent. of cod-liver oil and only 0·1 per cent. calcium carbonate had been added. On the other hand, raising the butter in the diet did not counterbalance a large dietetic deficiency of calcium. It may be added that it is strictly analogous results like this, embracing different physiological problems, which make it difficult to

accept the suggestion that the growth-promoting and the anti-rachitic actions of butter are due to separate entities.

In the following series of experiments it will be seen that the effectiveness of butter in promoting bone calcification is greatly increased when it works in conjunction with the remaining fraction of milk, and that this action of butter is not due to its fat content *qua* fat, because olive oil has but little effect under these conditions.

The Influence of Butter Fat and Separated Milk on Bone Calcification.—Their Relative and Combined Effects.

Experiments 530, 531, 532, 533, *and* 534.

The animals in this series were rapidly growing puppies.

Age at beginning of experiment: 8 weeks.

General daily diet eaten by all included: separated milk powder 15 gms., meat 10–20 gms., orange juice 3 c.cms., yeast 5 gms., bread up to 200 gms., sodium chloride 1 gm.

In addition, 530 received 30 gms. separated milk powder.
531 „ 30 gms. separated milk powder and 20 gms. butter.
532 „ 20 gms. butter.
533 „ 30 gms. separated milk powder and 20 c.cms. olive oil.

TABLE 8.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.* *Weeks.*	*Weight.* *Initial.* *gms.*	*Final.* *gms.*	*Max.* *gms.*	*CaO in Femur Shaft.* *Percentage.* *Dry.*	*Fresh.*	*Amount.* *gms.*	*Histology.*
530	+30 gms. sep. milk powder	21	1800	6050	7300 after 20 weeks	17·2	10·8	0·98	Bad rickets
531	+30 gms. sep. milk powder, 20 gms. butter	21	1870	8780	9000 after 20 weeks	22·2	15·8	1·89	Rickets
532	+20 gms. butter	21	2160	6500	6750 after 19 weeks	12·4	8·1	0·6	Very bad rickets
533	+20 c.cms. olive oil, +30 gms. sep. milk powder	21	2200	6750	8700 after 18½ weeks	16·1	11·0	1·15	Bad rickets, but healing
534	General diet only	20	1800	3280	4050 after 18 weeks	11·8	3·5	0·325	Very bad rickets, worse than 532

The points about the diets of these experiments worthy of comment are:

(1) In the diets of neither Exp. 534 nor 530 was there more than a trace of fat. The differences between the conditions of

534 and 530 were due to the extra separated milk eaten by 530.

(2) 532 and 534 received the same amount of separated milk, and differences between these animals were due to the 20 gms. of butter eaten daily by 532.

(3) 530, 531, and 533 received the same amount of separated milk powder, and comparison between 530 and 531 and 533 shows the relative influence of butter and olive oil when the calcium of the diet is fairly high.

(4) The rate of growth of these animals varied greatly, chiefly because of the difference in energy value and protein value of the respective diets (see Fig. 39).

The radiographs of the wrists of these animals, taken after 20 weeks of the experimental diets, are shown in Figs. 34, 35, 36, 37, and 38. It will be noted that all the animals of this series have developed rickets, as is evident by abnormality of the endochondral ossification. In the case of 531 (45 gms. separated milk powder and 20 gms. butter), Fig. 35, the rachitic changes are least intense. 533 (45 gms. separated milk + 20 c.cms. olive oil), Fig. 37, and 530 (45 gms. separated milk), Fig. 34, developed more severe rickets, but in the radiographs, especially Fig. 37, healing changes at the epiphyses are fairly advanced as the result of diminished appetite and loss of weight. The rachitic changes developed by the control animal 534 (15 gms. separated milk, no fat), Fig. 38, were the most severe in the series, but were healing in the radiograph. 532 (20 gms. butter), Fig. 36, had worse rickets than 530 and 531, but less intense than 534. In earlier radiographs, taken after 10 weeks of diet, 534 showed bad rickets, 530 and 533 rickets of equal intensity, 532 rather less rickets than the foregoing, while 531 was practically normal.

The calcium content of the bones set out in the table are in fair agreement with the radiographic evidence. Both separated milk and butter singly have brought about improvement in bone calcification. The former raised the calcium oxide in the femur shaft from 0·325 gm. to 0·98 gm., and the latter from 0·325 gm. to 0·6 gm. When the two acted together, as in Exp. 531, the calcium oxide increased to 1·89 gm. In Exp. 533, where the mineral salt content was practically identical with that of Exp. 531, the calcium oxide of the femur shaft was only 1·15 gm. It is possible from these figures to see how effective butter is in bringing about calcium deposition in the bones when this element is plentiful in the diet. Under the same conditions olive oil in the diet of Exp. 533 has had little or no effect in this respect.

Histological examination of the bones reveals very bad rickets in 534 (control) and 532 (butter only), bad rickets in 530 (separated milk) and 533 (separated milk + olive oil), and rickets in 531 (separated milk + butter). In view of the intensity of the calcification of the bones of 531 (separated milk + butter), as revealed by the high calcium content of the femur shaft, it is surprising that this animal has developed rickets. I drew attention to this effect, namely, the simultaneous development of rickets and good calcification produced by butter on bone, in a previous publication [1. *d*]. It may be

stated that these animals ate a large quantity of bread, and their weight increased rapidly (except Exp. 534), as can be seen in Fig. 39. It is apparent that in 531 the consumption of separated milk and butter has not completely antagonized the rickets-producing effect of the large amount of bread consumed. It will be further seen, in Fig. 39, that the additional fat eaten by 531 and 533 has brought about a greater increase in weight as compared with 530; also that the additional separated milk in the diets of 531 and 533 has increased their rate of growth as compared with 532. In the case of 534, where the diet contained little or no fat and only a small quantity of separated milk, the increase in weight was comparatively slow. In spite of the slow rate of growth, this animal developed the most severe rickets. It is evident, therefore, that increase in rate of growth due to the ingestion of separated milk is not associated with the added intensity in rachitic changes which accompanies the extra growth due to increasing the cereal of the diet.

The results obtained in these experiments, in which rapidly growing dogs were used, indicate that:

(1) Additional separated milk has had a definite but not a potent anti-rachitic action (cf. 530 with 534). It brought about better growth and well-being, but did not prevent severe rickets.

(2) Additional butter in the presence of a diet relatively deficient in some substance or substances present in milk (in the diet of 532 there were 20 gms. of butter and only about 150 c.cms. of milk) improved the calcification of bone and health of the animal, but did not prevent the development of severe rickets (cf. 532 and 534).

(3) Additional olive oil, even when the separated milk in the diet was high (i. e. 450 c.cms.), made but little difference to the calcification of bones, and did not prevent severe rickets (cf. 533 with 530).

(4) Butter added to a diet rich in separated milk had a great effect on the general condition of the animal, and improved greatly the calcification of the bones. In this case 531 there was still evidence of rickets at the epiphyseal ends of bone shafts, as observed by radiograph, taken in the latter part of the experimental period, but the periosteal bone was well calcified.

531 was a very active animal, and ran well throughout the experimental period. 530, 532, 533, and 534 became very feeble in the course of the experiment. Of these, 534 was in the worst condition.

The following experiments prove that the calcium element of the separated milk enhances greatly the anti-rachitic effect of the butter vitamin, but that separated milk has also some anti-rachitic effect, because of an additional constituent, probably a residue of anti-rachitic vitamin, remaining after the removal of its butter fat.

The Synergistic Action of the Anti-rachitic Vitamin in Butter and Calcium Carbonate.

Experiments 520–525.

In this series the animals were not of the rapidly growing type used in Exps. 530–534.

Age at beginning of experiment: 8 weeks.

General daily diet: separated milk powder 15 gms., meat 10 gms., bread 50–150 gms., orange juice 3 c.cms., yeast 5 gms., sodium chloride 1 gm.

TABLE 9.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial.	*Final.*	*Max.*	*Percentage.*		*Amount.*	
		Weeks.	*gms.*	*gms.*	*gms.*	*Dry.*	*Fresh.*	*gms.*	
520	General diet only	26	1550	3450	4250 after 20 weeks	13·6	7·9	0·44	Very bad rickets
521	+10 gms. butter	26	1470	4250	5240 after 20 weeks	15·7	10·8	0·66	Rickets
522	+20 gms. butter	26	1390	5850	6210 after 22 weeks	21·8	15·5	0·85	Rickets worse than 521
523	+20 gms. butter, 0·85 gms. $CaCO_3$	26	1970	5380	5380	23·3	16·8	1·32	Nearly normal
524	+0·85 gms. $CaCO_3$	25	1725	3880	5500 after 24 weeks	12·0	8·1	0·86	Bad rickets
525	+30 gms. sep. milk powder	26	1620	4700	5320 after 22 weeks	22·7	17·3	1·19	Nearly normal

It will be noticed in the above diets that:

(1) The diets of 520 and 525 are practically fat-free.

(2) If cow's milk be used as a standard, and 4 per cent. be regarded as its normal fat content, the fat eaten by 521, and more especially 522, overbalances the calcium of the diet. For instance, the 10 gms. butter eaten by 521 is equivalent to about 220–250 c.cms. of milk, while only the equivalent of about 150 c.cms. of separated milk was consumed. The discrepancy was even greater in 522, for 20 gms. of butter, equivalent approximately to that contained in 450–500 c.cms. cow's milk, were eaten, and the calcium of only 150 c.cms. of separated milk was included in the diet.

(3) The addition of 0·85 gm. of $CaCo_3$ to the diets of 523 and 524 brought the calcium content of the diets approximately to that of Exp. 525, where 450 c.cms. of separated milk was included in the ration.

The radiographic results (Figs. 40, 41, 42, 43, 44, and 45) of these animals after 20½ weeks of the diets showed that:

(1) 523 (Fig. 43) and 525 (Fig. 45) were practically normal animals. The bone calcification of both was good, and the animals were very active.
(2) 521 (Fig. 41) and 522 (Fig. 42) had definite rickets.
(3) 524 (Fig. 44) had rather worse rickets than 522 (Fig. 42).
(4) 520 (Fig. 40) had very bad rickets.

The calcium content of the bones is in agreement with the radiographic appearance of Figs. 40–45. 523 (butter + calcium carbonate) had much the most calcium oxide in the femur shaft (1·32 gm.), while the smallest amount (0·44 gm.) was present in the case of control animal 520. The additional separated milk eaten by 525 also improved the calcium deposition (1·19 gm.), and to a greater extent than additional calcium carbonate only (524, 0·86 gm.).

The histological appearance of the bones showed changes that would be expected from the radiographs and the calcium content.

The weight curves of these animals are given in Fig. 46. The slower increase in the case of 520 was due to the absence of fat from the diet. In the earlier part of the experiment, that is, for about 12 weeks, the weight of 520 increased fairly well, because the animal ate a good deal of bread and conserved its energy by reducing its activity. With the development of severe rickets its appetite diminished and the rate of growth became correspondingly smaller. The difference in the rate of increased weight between 522 (20 gms. butter) and 523 (20 gms. butter + $CaCO_3$) is not due to any difference between the protein or energy content of the respective diets, for these were identical except for the additional calcium carbonate eaten by 523. The diet of 522 made it a lethargic animal, while 523 was intensely active and lively. It is this difference in behaviour and metabolism of the animals resulting from abundant butter and calcium in the case of 523 that is probably responsible for its slower increase in weight as compared with 522. 523 (20 gms. butter + calcium carbonate) was the only animal that did not suffer loss of weight at the end of the experiment owing to diminished appetite.

The following deductions are suggested by these results:

(1) Comparing 520 with 524 the additional $CaCO_3$ of the diet has inhibited rickets but has not prevented it.
(2) Comparing 520 with 521 and 522, the butter, even in large quantities, has inhibited rickets and improved the bone calcification, but has not completely prevented the disease.
(3) Comparing 522 and 523, balancing the large amount of butter with additional $CaCO_3$ has prevented rickets and brought about much improvement in the latter animal's condition.
(4) Comparing 520, 524, and 525, it is clear that the better condition of 525 cannot be due only to the extra calcium in the separated milk, but that there are other factors which antagonize the disease, the most important of which is probably a certain amount of anti-rachitic vitamin still remaining when the butter fat is removed. If the extra

calcium in the separated milk were entirely responsible for the improvement of 525 over 520, then 524, which also received extra calcium in the form of calcium carbonate, ought to have been as good as 525, and this is not the case. It will be noticed that the additional separated milk has been more effective in preventing rickets in 525 than in 530 of the previously described series, although in the latter case also the separated milk had a definite anti-rachitic effect. The reason for the difference is undoubtedly that the 530–534 series of puppies were a more rapidly growing type and ate more bread.

The Antagonism of the Oatmeal Effect by Butter interacting with either Calcium Carbonate or Phosphate.

It will now be shown that the more potent rickets-producing effect of oatmeal can also be antagonized to some extent by a combination of butter and calcium carbonate or of butter and calcium phosphate. The action of these salts without the assistance of the anti-rachitic vitamin has been demonstrated in other experiments (see, for instance, Exps. 711, 713, and 715, Table 14, p. 33).

Experiments 748, 749, 751, *and* 752.

Age at beginning of experiment: 8 weeks.

The general diet eaten by all included separated milk powder 15–20 gms., meat 10 gms., oatmeal 30–80 gms., yeast 5 gms., orange juice 3 c.cms., sodium chloride 1–2 gms.

In addition, 748 received 10 gms. butter and 0·5 gm. calcium carbonate.

749 „ 10 gms. butter and 0·5 gm. calcium phosphate.

751 „ 10 c.cms. olive oil.

752 „ 10 gms. butter.

TABLE 10.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial.	*Final.*	*Max.*	*Percentage.*		*Amount.*	
		Weeks.	*gms.*	*gms.*	*gms.*	*Dry.*	*Fresh.*	*gms.*	
748	10 gms. butter, 0·5 gms. $CaCO_3$	17½	865	3460	3460	23·6	13·1	0·393	Rickets
749	10 gms. butter, 0·5 gms. $Ca_3(PO_4)_2$	17½	1000	2790	2910	25·8	14·0	0·452	Rickets
751	10 c.cms. olive oil	16	1350	2010	2420 after 7 weeks	15·6	6·8	0·164	Bad rickets
752	10 gms. butter	17	1200	1970	2890 after 15 weeks	17·3	8·7	0·274	Bad rickets

The radiographs (Figs. 47, 48, 49, and 50) taken at the end of the experiment, i. e. after 16–17 weeks of the dieting, show that both 751 (olive oil), Fig. 49, and 752 (butter), Fig. 50, have developed severe rickets, whereas 748 (butter and calcium carbonate), Fig. 47, and 749 (butter and calcium phosphate), Fig. 48, have much less rickets.

According to the histological appearance also, all the puppies of this series had developed rickets at the end of the experiments, but the pathological condition was more pronounced in 751 and 752 than in 748 and 749. The condition of 751 would undoubtedly have been even worse but for the fact that it soon lost appetite and ceased to put on weight after the first 6 weeks (Fig. 51).

After 14 weeks 752 (butter) also lost much weight. Cessation of growth of 751 (olive oil) is no doubt responsible for the fact that its radiograph (Fig. 49) indicates less severe rickets than 752 (butter), Fig. 50.

Comparing the calcium in the femur shafts of 751 with 752, it will be seen that butter only brought about an increase in calcium deposition from 0·164 gm. to 0·274 gm., but that when calcium carbonate and calcium phosphate were added to the diets in addition to the butter the calcium in the bone went up to 0·393 and 0·452 gm. in the respective cases.

It was again noticeable how relatively well calcified was the bone under the periosteum of the dogs receiving butter when contrasted with the defective calcification evident at the epiphyses. The calcium oxide content of the femur shaft also indicated good calcification, especially in 748 and 749, in spite of the rachitic changes which developed.

It would thus appear that the anti-rachitic action of butter, even when working in conjunction with additional calcium salts, has not been sufficiently potent to antagonize completely the rickets-producing effect of oatmeal in the above experiments. Butter, under these conditions, has not been nearly so effective as cod-liver oil in neutralizing the oatmeal action.

It is evident from the foregoing series of experiments that the anti-rachitic effect of milk depends partly on its butter fat and partly on the remainder of the milk. As regards the action of separated milk its calcium content is to some extent responsible for this, but there still remains unexplained a part of its anti-rachitic influence. The combined influence of butter fat and either a corresponding quantity of separated milk or of calcium carbonate or calcium phosphate in increasing bone calcification, and especially in respect of the point under discussion, in opposing the rickets-producing influence of oatmeal and other cereals, is relatively much stronger than would be expected from the effect of the butter fat only. Whole milk is much more strongly anti-rachitic than butter.

The problem as to why diets containing butter as the source of anti-rachitic vitamin often promote good calcification of the bone under the periosteum at a time when endochondral ossification appears very defective still remains unexplained.

(iii) *Green Vegetables.*

Until 1921, when a full report of this investigation was published, I had not been successful in carrying out experiments to test the anti-rachitic action of green vegetables on puppies. In view of the experimental work which had previously demonstrated the presence of vitamin A in these substances, it was obviously desirable that information as to their anti-rachitic properties should also be obtained, more especially because one of the main features of my work up to that point had been to compare and contrast the anti-rachitic and the growth-promoting properties of different foodstuffs. It may be remembered that, although the properties and distribution of the anti-rachitic substance and vitamin A were very similar, there were some differences which it appeared impossible to explain at the time. This subject has since formed the basis of many other investigations, a striking observation being that, whereas the vitamin A content of green vegetables (spinach), as tested by growth promotion of and the cure of ophthalmia in rats is large, their anti-rachitic effect is absent. McClendon and Shuck [5], for instance, found that 0·1 to 0·5 gms. of dried spinach cured ophthalmia but did not protect against rickets in rats, even when it formed 75 per cent. of the diet. Zucker and Barnett [6] also found that an alcoholic and ether extract of spinach did not protect against rickets in rats, but promoted their growth when the diet was otherwise devoid of vitamin A. Similar results were obtained with spinach by Goldblatt and Zilva [7]. Much of the work designed to solve this problem is open to the criticism that the basal diets used in the growth experiments and in the rickets experiments are different apart from the fat-soluble vitamin factor, and it seems to me that until the basal diets are the same in both types of work and complete, so far as is known, in all respects except as regards their fat-soluble vitamin content, no satisfactory solution of this problem can be reached.

The following experiments were carried out on puppies to test the anti-rachitic action of cabbage. It will be seen that this foodstuff has a definite effect in this direction. When oatmeal is the cereal eaten and the diet is otherwise deficient in anti-rachitic vitamin the influence of cabbage is small, but when bread replaces oatmeal the improvement in bone-formation is more obvious.

The Antagonistic Effect of Cabbage to the Rickets-producing Influence of Cereals.

Age at beginning of experiment: 10 weeks.

Diet eaten daily by both puppies included separated milk powder 20 to 25 gms., meat 20 gms., yeast 5 gms., orange juice 3 c.cms., olive oil 10 c.cms., sodium chloride 2–3 gms., and oatmeal 50–130 gms.

In addition 616 received cabbage 20–130 gms. daily. This was chopped up finely and boiled in water for 20 minutes before being added to the diet. The cabbage water was added to the diet.

The control diet eaten by 613 is potently rickets-producing, as can be seen in the radiograph, Fig. 52, taken after 17 weeks of the

diet. If the additional cabbage eaten by 616 had prevented rickets under these conditions its anti-rachitic effect might have been regarded as large. It will be seen, however, from Fig. 53, taken at the same time as Fig. 52, that this animal also developed severe rickets in spite of the cabbage ration, although the intensity of the disease is less than in the control animal 613. It will be noticed also that the calcium oxide in the femur shaft is much higher in 616 (cabbage) than in 613 (control), i. e. 0·93 gm. as compared with 0·62 gm. The animal eating cabbage was not only less rachitic but grew better, especially as regards its bones, and remained in a more active condition than the control puppy. The rate of increase in weight of these puppies can be seen in Fig. 54. It would seem probable from the more rapid increase in weight of 616 (cabbage) that this animal digested and assimilated a fair amount of the additional cabbage. The cabbage also delayed the onset of muscular paresis, for 613 could not run after 6 weeks of the diet, while 616 was not so affected until the ration had been eaten 14 weeks.

TABLE 11.

No. of Expt.	*Diet. Variable.*	*Duration of Expt.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial.	*Final.*	*Max.*	*Percentage.*		*Amount.*	
		Weeks.	*gms.*	*gms.*	*gms.*	*Dry.*	*Fresh.*	*gms.*	
613	Control	18	2850	6450	6570 after 17 weeks	12·7	4·1	0·62	Very bad rickets
616	Cabbage	17½	2950	7050	7120 after 16 weeks	14·7	8·8	0·93	Very bad rickets

When the rickets-producing influence of the diet is made less potent by substituting white flour for oatmeal as the cereal of the diet, then the anti-rachitic influence of cabbage is more prominent. In the following series of experiments the anti-rachitic influence of cabbage is contrasted with that of carrot, and both with egg yolk, white flour being the cereal eaten. It will be seen that the cabbage effect is greater than that of carrot, when equivalent weights are eaten, but that both actions are much less intense than that of egg yolk.

Comparative Effect of Cabbage, Carrot, and Egg Yolk.

Age at beginning of experiment: 8 weeks.

General diet daily eaten by all included separated milk powder 15 gms., meat 20 gms., yeast 5 gms., orange juice 3 c.cms., sodium chloride 1 gm., and bread 40 to 150 gms

In addition, 635 received 20 up to 120 gms. cabbage.

637 „ 20 „ 120 „ carrot.

638 „ 1/3 „ 1 egg yolk.

The cabbage and carrot were chopped up finely and boiled until cooked (20 minutes).

TABLE 12.

No. of Expt.	*Diet. Variable.*	*Duration of Expt. Weeks.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial. gms.	*Final. gms.*	*Max. gms.*	*Percentage. Dry.*	*Fresh.*	*Amount. gms.*	
635	Cabbage	33	1420	6400	6400	19·2	14·5	1·07	Rickets
637	Carrot	33	1580	6980	6980	13·8	9·2	0·75	Bad rickets
638	Egg-yolk	33	900	6290	6460	20·0	15·8	1·35	Nearly normal

Radiographs of these animals taken after 33 weeks of the experimental diets are represented in Figs. 55, 56, and 57. According to these, 638 (egg yolk), Fig. 57, is normal; 635 (cabbage), Fig. 55, has developed rickets, and 637 (carrot), Fig. 56, severe rickets.

The calcium results are in agreement, the animal getting some egg yolk daily having 1·35 gms. calcium oxide in its femur shaft, 635 (cabbage) having 1·07 gms., while 637 (carrot) has only 0·75 gms. According to the histological appearance 638 (egg yolk) was nearly normal; 635 (cabbage) had rickets, and 637 (carrot) had bad rickets.

The weight curves of these animals are given in Fig. 58.

It is clear that cabbage has had a distinct anti-rachitic action, but that even 120 gms. of it daily was not so effective as 1 yolk of egg. The anti-rachitic effect of the carrot was small and did not prevent the development of this disease in a severe form. It is, of course, particularly difficult to extend these results from dogs to other animals. In spite of the thorough cooking, a great deal of the carrot eaten was undigested, and this also applies to the cabbage. It would be expected, therefore, that, in those animals whose alimentary tracts are better able to deal with green and other vegetables, the anti-rachitic effect of these foodstuffs would be more potent. It is of interest, however, to note that, even in the case of a carnivorous animal like the dog, cabbage has some anti-rachitic action.

The anti-rachitic effect of egg yolk is very potent in its curative effect on experimental rickets, as I showed in an earlier publication [1. *d*], and as has been recently confirmed by Hess [8] on children. I have not tested its anti-rachitic action relatively to the rickets-producing effect of oatmeal, but the following experiment demonstrates as does the above described Exp. 638, that from a preventive standpoint it can antagonize the action of bread.

(iv) *Effect of Egg Yolk.*

Age at beginning of experiment: 8 weeks.

General diet daily eaten included separated milk powder 15 gms., meat 20 gms., bread 30–150 gms., orange juice 3 c.cms., yeast 5 gms., olive oil 10 c.cms., sodium chloride 1 gm.

604 ate this diet only.

606 also ate two-thirds to 1 egg yolk.

TABLE 13.

No. of Expt.	Diet. Variable.	Duration of Expt. Weeks.	Weight. Initial. gms.	Weight. Final. gms.	Weight. Max. gms.	CaO in Femur Shaft. Percentage. Dry.	CaO in Femur Shaft. Percentage. Fresh.	CaO in Femur Shaft. Amount. gms.	Histology.
604	Control diet	16	1350	3750	3750	21·7	14·0	0·60	Bad rickets
606	$\frac{2}{3}$–1 egg yolk	16	1090	4300	4300	27·4	16·7	0·86	Nearly normal

The radiographs of these animals taken after 16 weeks of the diet are produced in Figs. 59 and 60. It will be seen that 606 (egg yolk), Fig. 60, is normal, while 604 (control), Fig. 59, has developed rickets. These results are corroborated both by the histological examination and by the calcium oxide present in the bones. The weight curves of these animals are shown in Fig. 61.

The egg yolk not only brought about great improvement in the bones, but also in the general condition of 606. Whereas 605 (control) was weak and had some difficulty in moving about, 606 (egg yolk) was extremely active at the end of the experiment.

B. THE INTERACTION OF CEREALS WITH SALTS.

At an early stage of the discovery that cereals varied in their rickets-producing effect it was felt that the point as to whether the action was controlled by their calcium-phosphorus content had to be considered. This question is discussed at length at a later stage (see pages 46–50), but at this point a few experiments will be described which demonstrate how the cereal effect is influenced by altering the amounts and ratios of calcium and phosphorus in a diet deficient in anti-rachitic vitamin.

(i) *The Effect of varying the Calcium and Phosphorus Content of the Diet by adding Calcium Carbonate, Calcium Phosphate, and Sodium Acid Phosphate to Diets deficient in Anti-rachitic Vitamin.*

Age at beginning of experiment: 6 weeks.

General daily diet: separated milk powder 25–30 gms., meat 10–20 gms., sodium chloride 1–4 gms., yeast 3–5 gms., orange juice 3–5 c.cms., oatmeal 40 to 100 gms.

As regards the above general diet two points are to be noticed: (1) the cereal used throughout was oatmeal, (2) the separated milk powder in the diet was moderately large from the beginning of the experiment, i. e. 25 gms., and was soon raised to 30 gms. daily. The object of giving more separated milk than in many of the other experiments was to antagonize to some extent the very potent rickets-producing effect of the oatmeal and so, by prolonging the experimental period during which the animals ate their food quantitatively, to obtain better comparative results.

In the following series of experiments the calcium and phosphorus

content of the food was altered by adding calcium carbonate, calcium phosphate $Ca_3 (PO_4)_2$, and sodium acid phosphate $NaH_2 PO_4$ respectively, to the general diet of three of the four animals.

TABLE 14.

No. of Expt.	Diet. Variable.	Duration of Expt. Weeks	Weight. Initial. gms.	Weight. Final. gms	Weight. Max. gms.	CaO in Femur Shaft. Percentage. Dry.	CaO in Femur Shaft. Percentage. Fresh.	CaO in Femur Shaft. Amount. gms.	Histology.
713	Control	22	2430	5510	5590 after 17 weeks	14·2	5·7	0·51	Very bad rickets
715	+ 0·37 to 0·74 gms. $CaCO_3$	23	2220	6450	6450	11·4	7·6	0·98	Bad rickets
711	+ 0·38 to 0·76 gms. $Ca_3(PO_4)_2$	22	2230	4430	5830 after 18 weeks	15·8	6·5	0·63	Very bad rickets
710	+ 0·3 to 0·6 gms. NaH_2PO_4	22*	2280	—	—	—	—	—	—

* N.B.—After 22 weeks diet changed and animal used for other purposes.

The calcium in the added calcium carbonate (0·37 to 0·74 gm.) and calcium phosphate (0·38 to 0·76 gm.) is equivalent to that present in about 125 to 250 c.cms. of cow's milk, so that, from the point of view of calcium content, the diets of 715 and 711 can be considered as having an equivalent of not less than 370 to 550 c.cms. of cow's milk. The larger quantity was taken throughout most of the experimental period.

The additional phosphorus in the diets of 711 (0·38 and 0·76 gm. $Ca_3(PO_4)_2$) and 710 (0·3 to 0·6 gm. NaH_2PO_4) is equivalent to that present in 80 to 160 c.cms. of milk.

The calcium in the diets of 715 ($CaCO_3$) and 711 ($Ca_3(PO_4)_2$) is identical, and the phosphorus in the diets of 711 ($Ca_3(PO_4)_2$) and 710 ($NaH_2 PO_4$) is also equal.

The figures in Table 15 represent the lower and upper limits of calcium and phosphorus intake of these animals during the experimental period. The lower figures in each case show the amount supplied in the food during the first two weeks of the diet, and the higher figures from that time until the rachitic condition was severe and less food was eaten, i. e. about 14 weeks, after which time the amount ingested was reduced equally in each case.

The radiographs of the wrists of these animals taken after 13 weeks of the diet can be seen in Figs. 62, 63, 65, and 67. 710 (sodium acid phosphate), Fig. 62, and 713 (control), Fig. 65, have developed most severe rickets, and to a similar degree of intensity, while 715 (calcium carbonate), Fig. 67, is the least rachitic of these four animals. 711 (calcium phosphate), Fig. 63, has bad rickets, but not as severe as 710 and 713. The effect of adding calcium carbonate to the rickets-producing diet appears from these radiographs (Figs. 65 and 67) to be great, but it will be seen in Figs. 70 and 72, a radio-

graph and photograph of 715 (calcium carbonate) taken at a later stage, namely, after 22 weeks of the diet, that this animal also developed fairly severe rickets. As regards sodium acid phosphate, it is evident that it has not brought about any increased resistance to the disease. Calcium phosphate (Fig. 63), on the other hand, has had a definite but not great anti-rachitic action under these conditions.

TABLE 15.

	Ca Intake.	*P intake.*	$\frac{Ca}{P}$
710	0·350 to 0·477 gms.	0·480 to 0·850 gms.	1 : 1·76
711	0·498 to 0·772 gms.	0·480 to 0·850 gms.	1 : 1·10
713	0·350 to 0·477 gms.	0·411 to 0·701 gms.	1 : 1·47
715	0·498 to 0·772 gms.	0·411 to 0·701 gms.	1 : 0·91

The calcium content of the bones is in agreement with the radiographic results. The calcium oxide in the femur shaft has been raised by the additional calcium carbonate in the diet from 0·51 gm. to 0·98 gm., and by calcium phosphate from 0·51 to 0·63 gm. Parenthetically, it may be well to point out how small these figures are compared with the 1·53 gm. of calcium oxide in the femur shaft of Exp. 716, a member of the same litter having the same diet as the control (713) except that cod-liver replaced olive oil.

The rate of increase in weight of these animals is given in Fig. 80. For the first 9 weeks of the experiment the diets were well eaten and the increase in weight in all cases was similar. After this period, and especially after the twelfth week, 713 (control), 710 (sodium acid phosphate), and 712 (calcium phosphate), having developed severe rickets, no longer finished their ration, so that their weight did not increase so rapidly. The diet of 715 (calcium carbonate) was cut down in an attempt to keep it parallel with 713, 710, and 712, but even so it put on more weight than the others.

Although it is evident that the rickets-producing effect of oatmeal can be modified by alteration in the amount and proportion of calcium and phosphorus in diets deficient in anti-rachitic vitamin, this method of defence is limited in its potency, for all these animals developed rickets in spite of the variation in the intake of these elements. It would appear doubtful, therefore, whether the oatmeal effect can be explained by its calcium-phosphorus content or even whether a first line of defence against the effect of cereals is worth seeking in the calcium-phosphorus contents of a diet. Thus when the effect of adding these salts to the basal diet is studied it will be seen that:

(1) The additional calcium carbonate has had a distinctly beneficial effect. The bones are better formed than those of the control, and indeed than those of the dog eating additional calcium phosphate. It may be added that the animal was obviously better

for the extra calcium carbonate and throughout the experimental period was more active and in better condition.

(2) The addition of calcium phosphate has retarded the rachitic condition to a slight degree only, in contrast to the calcium carbonate effect. In an earlier publication I showed that calcium phosphate added to a rickets-producing diet did not prevent rickets. While this is true it would appear to have a slight anti-rachitic action in the above described and in other experiments of a similar nature which have been carried out.

(3) The addition of sodium acid phosphate seems to have had no anti-rachitic effect.

(ii) *The Action of Calcium Hydrogen Phosphate,* $CaH_4(PO_4)_2$, $2H_2O$.

The following experiments are described partly because they illustrate the action of a combination of calcium and phosphorus different from those described elsewhere in this work, and partly because the separated milk was by degrees eliminated from the diets, so that almost all the calcium ingested was provided in the form of this salt to some of the animals, while in other cases the diet remained almost devoid of calcium. It was a matter of interest to see whether the animal body could make use of and retain adequately in its growing bones the calcium supplied in this form both when the anti-rachitic vitamin was abundant and when it was deficient. Calcium hydrogen phosphate is fairly soluble—unlike calcium carbonate and phosphate—and it is of acidic reaction.

Experiments 492, 494, 495, *and* 496.

Age of puppies at beginning of experiment: $6\frac{1}{2}$ weeks.

General daily diet eaten by all.—Separated milk powder started at 10 gms. daily and was gradually reduced so that at the end of 7 weeks none was eaten, i.e. for $21\frac{1}{2}$ weeks of the experimental weeks no separated milk was included in diet; meat 10–30 gms., bread 30–170 gms,, sodium chloride 1 gm., orange juice 3 c.cms., and during the last 17 weeks 5 gms. of yeast daily.

In addition, 492 received 3 c.cms. linseed oil

494	,,	,,	linseed oil + 1 gm. $CaH_4(PO_4)_2$, $2H_2O$
495	,,	,,	cod-liver oil
496	,,	,,	cod-liver oil + 1 gm. $CaH_4(PO_4)_2$, $2H_2O$.

The diet of 492 was very deficient both in anti-rachitic vitamin and calcium, 494 was very deficient in anti-rachitic but contained some calcium, 495 had only traces of calcium but a fair supply of vitamin and 496 had both anti-rachitic vitamin and calcium.

The daily intake of calcium and phosphorus was somewhat as follows:

		492 *and* 495	494 *and* 496
Average amount during first 7 weeks	Calcium	0·085 gms.	0·225 gms.
	Phosphorus	0·165 ,,	0·395 ,,
,, ,, ,, last $21\frac{1}{2}$,,	Calcium	0·045 ,,	0·183 ,,
	Phosphorus	0·205 ,,	0·434 ,,

TABLE 16.

No. of Expt.	Diet. Variable.	Duration of Expt. Weeks.	Weight. Initial. gms.	Weight. Final. gms.	Weight. Max. gms.	CaO in Femur Shaft. Percentage. Dry.	CaO in Femur Shaft. Percentage. Fresh.	CaO in Femur Shaft. Amount. gms.	Histology.
492	3 c.cms. linseed oil	21½	785	2500	2750 after 16 weeks	Bones too soft to deal with		—	Very bad rickets
494	3 c.cms. linseed oil + 1 gm. $CaH_4(PO_4)_2,2H_2O$	28½	1500	3640	4550 after 25 weeks	13·6	7·9	0·64	Very little calcification Bad rickets, but better than 492
495	3 c.cms. cod-liver oil	28½	900	3900	3900	20·8	11·2	0·44	Very slight rickets but osteoporosis
496	3 c.cms. cod-liver oil + 1 gm. $CaH_4(PO_4)_2,2H_2O$	28½	1295	4450	4450	17·8	13·0	0·88	Nearly normal

Radiographs (not reproduced) of these animals, taken during the experimental period, show that the rachitic changes are intense in the case of 492 (−vitamin and −calcium), less intense in 494 (+calcium −vitamin), while the epiphyseal growth in 495 (−calcium + vitamin) and 496 (+calcium +vitamin) shows but little derangement.

The illustrations of the experimental results in this series of experiments are given in the form of photomicrographs of the costo-chondral junctions (Figs. 81, 82, 83, and 84). These sections were not decalcified but, after fixing in Müller's solution, were cut by freezing microtome and stained direct in silver nitrate and eosin. The intense black portions of the figures represent bone calcium. It will be seen that there is practically no calcium in the bones of 492 (−calcium −vitamin), Fig. 81, while in 496 (+calcium +vitamin), Fig. 84, the calcification is fairly intense. An attempt at healing with renewed calcification is evident in 492 (Fig. 81). This is no doubt due to loss of appetite and diminution in weight towards the end of the experiment. In 495 (−calcium +vitamin), Fig. 83, it would appear that a great effort is being made to keep the actual calcification at the epiphyseal growing margin in working order, but that this is being done at the expense of calcium deposited in the older bony trabeculae and in the portion of the shaft previously formed. In 494 (+calcium −vitamin), Fig. 82, there is a moderate amount of calcium in the costo-chondral junction, but great disorder in the growing margin. There is also a fair amount of osteoid tissue present in 494 but very little in 496.

The bones of 492 (−vitamin and −calcium) were so soft and devoid of calcium that it was impossible to dissect out the femur shaft intact for chemical analysis. The calcium oxide contents of the bones in the other experimental animals of this series are instructive. For instance, in spite of the low calcium content in the case of 495 (−calcium +vitamin) (0·44 gm.), there was no rickets but only severe osteoporosis. The presence of calcium acid phosphate in the diet of 494 brought about an increase in calcium

salts in the bones (0·64 gms.), but did not prevent severe rickets with great derangement of structure. The added calcium and phosphate in the presence of vitamin (496) resulted in more intense bone-calcification (0·88 gm.) and no rickets.

It may be inferred from these results that, when flour was the cereal eaten, (1) calcium in the diet in the form of calcium acid phosphate was retained and brought about great improvement in the animal's condition, but did not prevent severe rickets when the anti-rachitic vitamin of the diet was deficient; (2) cod-liver oil in small quantities (3 c.cms. daily) prevented rickets but not osteoporosis when the calcium intake was negligible: when calcium acid phosphate was also added to the diet normal bones and a healthy animal developed; (3) when both calcium and anti-rachitic vitamin were absent the bones became osteoporotic and rachitic.

Except in the case of sodium phosphate, where the results have been somewhat contradictory, the experiments above described dealing with the action of calcium and phosphorus-containing salts are typical of those obtained in other series, for whenever it has been tested calcium carbonate has had a distinct anti-rachitic action and calcium phosphate and calcium acid phosphate a slighter effect in the same direction. In one or two experiments the calcium carbonate effect has been so great as to result in the production of almost normal bones, but in most cases rickets has developed but to a less degree than in the control animals not receiving additional chalk. Why there should be this variation is not known, but the animals in which the anti-rachitic action of the calcium carbonate has been most potent have been small animals eating less food and less liable to develop rickets than the heavy type of puppy. There seems, however, to be but little doubt that the close interaction of the anti-rachitic vitamin and calcium allows a certain amount of interchange between the relative amounts of these substances, so that in some circumstances a low anti-rachitic vitamin content of a diet may be counterbalanced by a high calcium content. I have previously pointed out how much more effective the anti-rachitic vitamin of butter is in the presence of additional calcium salts, and that under this condition calcium phosphate appears to be as effective as calcium carbonate (see Exps. 648 and 649). Other experiments, not described here, have demonstrated that calcium acid phosphate also is more completely incorporated in the body when the diet contains butter.

It is necessary to emphasize how relatively small is the effect of adding calcium carbonate or calcium phosphate only to a diet deficient in anti-rachitic vitamin as compared to the addition of a good source of this vitamin, such as cod-liver oil or egg yolk, or to a combination of butter fat and these salts, such as milk. In order to illustrate this point, I have placed side by side radiographs and photographs of two puppies of the same litter both on the same basal diet, one of which had received extra calcium carbonate daily (Figs. 70 and 72) and the other cod-liver oil (Figs. 71 and 73). It will be at once evident how much more effective is cod-liver oil in bringing about improvement in deposition of calcium salts in the bones than additional calcium in

the diet. The better calcification and formation of the bones was associated with greater activity, liveliness, and better health of the animal, so that in every way the added source of vitamin was superior to the calcium carbonate.

The question arises as to whether the calcium carbonate exerts its anti-rachitic effect (1) by neutralizing the rickets-producing action of oatmeal, (2) by increasing the effectiveness of any anti-rachitic vitamin there may be in the diet, or (3) by both of these actions. The dietetic conditions responsible for the production of rickets are so closely bound together and interdependent that it is impossible at this stage to give a definite answer to the above questions. One of the experimental conditions which would have to be fulfilled before a definite answer could be given would be the complete elimination of the anti-rachitic vitamin from the diet. In these experiments there has always been a small amount of this vitamin present, even in the most rickets-producing diets used. It can, however, be definitely stated that calcium carbonate improves, to some extent, bone-calcification, even when the anti-rachitic vitamin in the diet is small.

Summary of Section IV.

Whereas it is clear from the above experiments that foodstuffs rich in anti-rachitic vitamin oppose to different degrees the rickets-producing effect of cereals, the outcome of the contest obviously varies greatly according to many circumstances, including the source and amount of vitamin in the diet and the type and amount of cereal used.

Cod-liver oil is so potently anti-rachitic that it can easily antagonize the rickets-producing action of cereals, even of oatmeal. Egg yolk also acts powerfully in overcoming the cereal effect. Butter alone exerts little antagonism to the cereal, but, working in conjunction with the remaining constituents of milk, its anti-rachitic influence is greatly enhanced. When the calcium content of the diet is raised by adding calcium carbonate or calcium phosphate, the calcifying action of the vitamin in butter is much increased. The anti-rachitic action of separated milk is definite, but cannot be explained solely by its calcium content. Whole milk is more intensely anti-rachitic than the influence of its butter fat content would suggest. Cabbage, under the experimental conditions described, has a definite but relatively feeble effect in combating the influence of oatmeal. It is possible the green vegetable action would be much greater if the tests were made on herbivorous animals than when made on puppies, whose alimentary canal is obviously ill-adapted for the digestion of these foodstuffs. Calcium carbonate, calcium phosphate, and calcium acid phosphate increase the deposition of calcium salts in bone and antagonize, to some extent, the rickets-producing effect of cereals, even when the anti-rachitic vitamin in the diet is small. Calcium carbonate is the best of the salts tested under these conditions.

V. THE INTERACTION OF CEREALS WITH ULTRA-VIOLET RADIATIONS.

A. Exposure of the Skin to Ultra-Violet Radiations.

The curative effect of exposure of the skin to ultra-violet radiations of a mercury-vapour lamp on human rickets was demonstrated by Huldschinsky [12 *a*] in 1919, and since that date other investigators have published confirmatory observations on this subject. Besides the clinical confirmation of these facts by Hess and Unger [9 *b*], and also by Chick, Dalyell and colleagues [13], the matter became a subject of experimental study on animals, more especially on rats. These animals were placed on rickets-producing diets, which were modifications of a type used by Sherman and Pappenheimer [16 *a*], and were exposed at intervals during the experimental period to the radiations emitted either by the sun or by lamps of various forms. The essential characteristics about these diets were that both the fat-soluble vitamin and the phosphorus were deficient. Under these dietetic conditions it was found by Hess, Unger, and Pappenheimer [10] on the one hand, and Shipley, Park, Powers, McCollum, and Simmonds [14] on the other, that exposure to ultra-violet radiations had a strong anti-rachitic effect. Huldschinsky [10 *b*] had also found that the action of the radiations was not confined to improvement in bone-calcification of rachitic children, but that in addition they exerted a curative influence in tetany. In the case of the rat experiments Powers, Park, Shipley, McCollum, and Simmonds [15] found that the sunlight also brought about a greater consumption of food, stimulated activity, improved the appearance, and increased the reproductive capacity. The light influence on the metabolism was, in fact, widespread.

Somewhat earlier than the publications of Huldschinsky it had been found that diet played an important part in the aetiology of rickets, and that in particular a substance similar in many properties and in distribution to vitamin A had a potent controlling influence on bone-calcification (E. Mellanby 1 *a*, *b*). It became a matter of interest to see in what way the respective influences of diet and exposure to ultra-violet radiations were related to each other so far as the calcification of bone was concerned. The suggestion was made by the American workers that the action of radiation was similar to adding phosphorus to the diet (Hess and Gutman [11], Powers, Park, Shipley, &c. [15]). This suggestion seemed feasible when the experimental results on rats were being considered, but had no meaning from the point of view of human rickets where there was no reason to believe that a dietetic phosphorus deficiency was of importance. It soon became apparent that the effects of ultra-violet radiation were closely similar to those produced by adding a source of vitamin A to a diet previously deficient in this substance. The question then arose as to whether the radiations, striking the skin, brought about the synthesis of this vitamin or that they activized the supplies of this substance already present in the body. It is now probable, as the result of the experimental work on the growth of

rats by Hume [17] and also Goldblatt and Soames [18], that the radiations mobilize and stimulate to activity the supplies of fat-soluble vitamin already present, and that they do not increase its amount. It has not yet been demonstrated that in the case of bone-calcification, also, the action of ultra-violet rays depends upon stimulating to activity the anti-rachitic vitamin supplies in the body, but the similarity in distribution and behaviour between the substances stimulating growth of young rats and the calcification of bone respectively is so close that it is most probable that this explanation can be extended to the anti-rachitic influence of the radiations. Since the only means of access of anti-rachitic vitamin to the body, so far as is known, is by the food, except in the unborn and suckling infant, where the origin must be through the maternal food, it is clear that the interplay and interdependence between the fat-soluble vitamins in the food of an animal and the exposure of its skin to sunlight or other source of ultra-violet radiation must be of great significance as regards the formation of bone and other tissues. In fact the experiments indicate that, under those conditions which allow exposure of the body to sunlight, less anti-rachitic vitamin in the food will be compatible with perfect bone-calcification than when there is no chance of such exposure: that is to say, the anti-rachitic vitamin of the diet can be to some extent replaced by exposure to ultra-violet rays.

In view of the facts described in this publication, it ought to be possible to extend this question of the interaction of food and sunlight, for it has been shown that there is in cereals, and especially in oatmeal, a substance which, instead of aiding the action of the anti-rachitic vitamin, actively antagonizes it. On the basis of interchangeability (within limits) between the anti-rachitic vitamin of food and exposure to ultra-violet radiations, it would be expected that the substance in cereals under study would also antagonize the influence of the ultra-violet rays, just as it antagonizes the vitamin. Experiments will now be described which show that this is the case, so that, when the diet is deficient in anti-rachitic vitamin, the effect of exposure to the radiations is large only when small quantities of cereal are eaten, and that as these increase, especially if in the form of oatmeal, the smaller becomes the calcifying influence of the rays.

The Antagonistic Action between Exposure of the Skin to Ultra-Violet Rays and Oatmeal in the Food.

Age at beginning of experiment: 6 weeks.

General daily diet, eaten by all the puppies, consisted of separated milk powder 25–30 gms., meat 10–20 gms., orange juice, 3–5 c.cms., yeast 5 gms., sodium chloride 1–4 gms. and olive oil 10 c.cms.

In addition, 713 received 60–100 gms. oatmeal.

717	„	60–100 gms.	„
712	„	30–50 gms.	„
714	„	30–50 gms.	„

The abdominal regions (after shaving) of 717 and 714 were exposed to the radiations of a mercury-vapour lamp at a distance of

about 1 yard for a period of 30 minutes thrice weekly throughout the experimental period.

TABLE 17.

No. of Expt.	Diet. Variable.	Exposure to Radiations.	Duration of Expt. Weeks.	Weight.			CaO in Femur Shaft.			Histology.
				Initial. gms.	Final. gms.	Max. gms.	Percentage. Dry.	Percentage. Fresh.	Amount. gms.	
713	2 *x.* Oatmeal	0	22	2430	5510	5590 after 17 weeks	14·2	5·7	0·51	Very bad rickets
717	2 *x.* do.	+	22	2330	5720	5750 after 20 weeks	14·8	7·5	0.79	Bad rickets
712	*x.* do.	0	22	2140	3860	4430 after 16 weeks	26·4	7·7	0·68	Bad rickets
714	*x.* do.	+	22	1900	4700	4700	22·6	11·8	1·14	Rickets

The results of these experiments, and particularly the radiographs (see Figs. 65, 69, 64, and 66), show clearly that exposure to the ultra-violet rays has resulted in a definite anti-rachitic effect and an improvement in calcification. There is, however, a great difference in the amount of improvement brought about in Exp. 714 (Fig. 66) as compared with 717 (Fig. 69). This is also evident in the photographs of sections of the costo-chondral junctions of these animals, 713, 717, 712, and 714, which are shown in Figs. 75, 79, 74, and 76. It will be seen that the junction of 714 (Fig. 76) is much more normal than 712 (Fig. 74). On the other hand, that of 717 (Fig. 79) is somewhat similar in rachitic derangement to 713 (Fig. 75). In 712 and 714 the rickets-producing effect of the diet was relatively small, because, although the diet was deficient in anti-rachitic vitamin, the amount of cereal eaten was moderate in amount. In 713 and 717 the cereal eaten was double that in the diet of 712 and 714, so that the rickets-producing effect of the food was great. When the rickets-producing effect of the diet was small the effect of the radiations on bone-calcification was large, so that, for instance, the shaft of the femur of 714 contained 1·14 gms. of calcium oxide as compared with 0·68 gm. in 712. The difference, 0·46 gm. CaO, can probably be ascribed to the effect of the ultra-violet light. When the rickets-producing effect of the diet was large the effect of the radiations was less pronounced, for whereas the femur shaft of 713 (control) contained 0·51 gm. CaO, that of 717, which received light treatment but the same diet, contained only 0·79 gm. CaO, an increase of 0·28 gm. as compared with the increase of 0·46 gm. calcium oxide in 714, although the same exposure to light was experienced in each case. The normal dog in this litter (716) which received cod-liver oil instead of olive oil had 1·53 gms. calcium oxide in its femur shaft. The histological appearance of the bones is in harmony with their radiographic appearance and chemical composition.

Weight curves showing differences in the rate of increase in

weight according to the amount of oatmeal in the diet are given in Fig. 80. It will be seen that 713 and 717 ($2x$ oatmeal) put on weight more rapidly than 712 and 714 (x oatmeal).

Two other series of experiments dealing with the question of interaction between light radiations, diet, and bone-calcification were carried out, but will not be referred to in detail here. In one set of puppies the experimental methods were similar to those already described, but the worst diets were even more unbalanced than in 713 and 717 described above. In this series the separated milk in the diet amounted to 150 c.cms. as compared with the 250 to 300 c.cms. taken by 713 and 717. Exposure to the mercury-vapour lamp greatly improved the bone-calcification and general condition when the cereal in the diet was kept small, but with much oatmeal eaten exposure to the rays brought about no improvement.

In a third series the control animals were kept indoors and the others were exposed to the sunshine in the open. These experiments were carried out in May and June, 1922, when there was a good supply of sunlight. In addition to the sunlight the exposed puppies had other advantages, for they had more opportunity for exercise, more fresh air, and a different temperature. The results, however, were in general similar to those obtained with the mercury-vapour lamp. When the oatmeal was kept small the exposed animal was much more active and had better calcified bones than the corresponding animal indoors, but, with a large intake of oatmeal, when the diets were also deficient in anti-rachitic vitamin, the sunlight and other factors brought about no detectable improvement.

It is evident that the more oatmeal in the diets above described the worse is the rickets produced and the less effective is the exposure to the mercury-vapour lamp; also that just as there is an antagonism between cereal and anti-rachitic vitamin in the food so far as rickets and general nutrition is concerned, so there is an antagonism between the rickets-producing substance of cereals and exposure of the animals to ultra-violet radiations.

B. Exposure of Oatmeal to Ultra-Violet Radiations.

It has been found recently by Steenbock and Black [45] and also by Hess [46] that exposure of certain foodstuffs devoid of fat-soluble vitamins to ultra-violet radiations confers upon these substances nutritive properties similar in many respects to those of the absent vitamins. Thus a synthetic diet, perfect so far as is known except for a deficiency of growth-promoting and anti-rachitic fat-soluble vitamins, acts temporarily after exposure to ultra-violet radiations as if containing these specific substances. In fact it seems to matter but little whether the radiations are applied to the skin of the experimental animal (rat) or to the vitamin-deficient food eaten by the animal.

Even irradiation of the sawdust, used as bedding in cages inhabited by rats eating a diet deficient in fat-soluble vitamins, confers upon these animals powers of growth similar to those possessed by directly irradiated animals eating a similar diet. (Hume and Smith [48].)

It is difficult to comment upon these experimental results, for the question of inter-relationship between nutrition, vitamins, and radiations is both new and unexpected and knowledge of the subject is but meagre. It is first necessary to accumulate facts concerning the phenomena, and, among other things, to determine the relative importance and potency of the different factors. One point seems probable both as regards the effect of radiations on the skin and on the food, namely, that these do not synthesize or bring about the formation of fat-soluble vitamins but stimulate to activity those vitamins present in the body.

It has been shown above that the rickets-producing effect of oatmeal can be antagonized both by the anti-rachitic vitamins in food and to a less extent by the application of ultra-violet radiations to the skin. It seemed of interest to know whether the direct exposure of oatmeal to ultra-violet radiations would have a similar effect and so minimize some of its toxic action. The following experiments indicate that this is the case.

The Exposure of Oatmeal to Ultra-Violet Radiations.

Experiments 877, 878, 879, 880, and 881.

Age at beginning of experiment seven weeks.

General daily diet eaten by 877, 879, 880, and 881 consisted of separated milk powder 20 gms., meat 10 gms., olive oil 10 c.cms., yeast 5 gms., orange juice 3 c.cms., sodium chloride 1 to 3 gms., and oatmeal 30 to 100 gms.

The diet of 878 was the same except that 1 c.cm. of olive oil was replaced by 1 c.cm. of cod-liver oil, thus allowing the effect of the radiations to be compared with that of a small amount of a definitely anti-rachitic substance.

Some ingredients of the diets were exposed to the radiations of a mercury-vapour lamp as follows:

877—oatmeal was radiated before cooking.

879—oatmeal was first cooked and then exposed to radiations.

880—olive oil was exposed to radiations.

878 and 881—none of the food was radiated.

The foodstuffs exposed to the ultra-violet radiations, i.e. oatmeal (877), porridge (879), and olive oil (880), were spread out in thin layers in photographic developing dishes and were placed within two feet of the mercury-vapour lamp. The exposure lasted for thirty minutes, during which time the substances were often stirred to allow a large surface being influenced. A two days', and on Saturdays, a three days' supply of the ingredients was exposed at one time. In the case of 877 the oatmeal was cooked in a steaming utensil for 1½ hours after exposure to the radiations, whereas in the case of 879 the cooking preceded the application of the radiations.

The radiographs taken after six weeks of the experimental treatment are shown in Figs. 97, 98, 99, 100, and 101. It will be seen that 881, Fig. 101, the control animal which had none of its food radiated, has developed severe rickets, whereas 877, Fig. 97 (oatmeal radiated), and 879, Fig. 99 (porridge radiated), only show slight and similar rickets. 880, Fig. 100 (olive oil radiated), is

nearly normal, 878, Fig. 98 (1 c.cm. cod-liver oil), is normal. It is necessary to state that, although 877 (oatmeal radiated) has developed but slight rickets even according to X-ray examination, made at a later stage than that of Fig. 97, it shows great muscular weakness. This combination of slight rickets and great muscular weakness is unusual. In the case of 881 (no radiations) the animal has developed muscular weakness and severe rickets, which is usual with this diet.

No. of Expt.	*Diet. Variable.*	*Duration up to time of radiograph. Weeks.*	*Initial weight. Gms.*	*Weight at time of radiograph. Gms.*	*Radiographic results* (see Figs. 97, 98, 99, 100, and 101).
877	Oatmeal radiated	6	1460	2860	Commencing Rickets
878	1 c.cm. of cod-liver oil. No radiations	6	1440	2850	Normal
879	Oatmeal cooked and radiated	6	1580	3150	Commencing Rickets
880	Olive oil radiated. Oatmeal not radiated	6	1500	3020	Commencing Rickets
881	No radiations	6	1800	3170	Bad Rickets

Up to this stage of the experiment all the diets were eaten completely. The weight curves are shown in Fig. 102.

It appears from these results, which are however only of a preliminary nature, that exposure of oatmeal, cooked or uncooked, to ultra-violet radiations of the mercury-vapour lamp has so influenced the cereal that its rickets-producing power has been reduced. Similarly, exposure of the olive oil of the diet has conferred upon it anti-rachitic properties tending to inhibit the oatmeal effect. Cooking the oatmeal after irradiation did not destroy the effect of the exposure. It is important to notice that, striking as these results of food irradiation are, the anti-rachitic effect of the diet conferred by irradiation as carried out in these experiments is smaller than that exerted by one cubic centimetre of cod-liver oil. In the appraisement of the practical value of ultra-violet radiations, so far as one aspect of their physiological influence is concerned, this observation is of importance.

Result: From preliminary experiments it would appear that some part of the rickets-producing influence of oatmeal can be eliminated by direct exposure of this cereal, either cooked or uncooked, to ultra-violet radiations of a mercury-vapour lamp. The same kind of antagonism is seen as when the animals eating oatmeal as an ingredient of a diet deficient in anti-rachitic vitamins are themselves exposed to ultra-violet rays.

VI. DISCUSSION OF THE MODE OF ACTION OF CEREALS IN PRODUCING RICKETS.

The evidence given above shows that, under the experimental conditions described and especially when the diet is deficient in anti-rachitic vitamin, (1) increasing the cereal in the diet increases the intensity and hastens the onset of rickets in puppies, and (2) varying the type of cereal in the diet of puppies, while keeping all other conditions constant, results in great differences in the intensity of the disease. It has also been shown that the cereal rickets-producing action can be antagonized or modified by (1) the presence in the diet of foodstuffs containing the anti-rachitic vitamin, such as cod-liver oil, milk, egg yolk, cabbage, &c., (2) exposure of the skin or the cereal to ultra-violet rays, which probably act by mobilizing and activizing latent stores of anti-rachitic vitamin in the body and (3) the presence of salts containing calcium and phosphorus in the food.

It is necessary now to inquire into the explanation of these results, and more especially to seek the reason for the different rickets-producing influences of the various cereals. Why should oatmeal be more potently rickets-producing than rice or white flour, and why should wheat germ intensify the disease? It is obviously necessary in the first place to try to find a solution to this problem in terms of known constituents of cereals and, it is from this point of view, that the experimental results will now be considered.

(A) The Carbohydrate Moiety of Cereals.

Partly because carbohydrate is quantitatively the most important constituent of cereals and partly because this substance is often held responsible for the production of rickets in children, it is but right that this aspect of the question should be first examined.

I pointed out earlier in this publication that the experiments in which pure carbohydrate was added to the diet were difficult to carry out, and that in the series of experiments which were fairly satisfactory the results indicated that carbohydrate alone did not appear to explain satisfactorily the total effect of varying quantities of bread in the diet. This conclusion is confirmed most strongly when the carbohydrate content of the different cereals whose rickets-producing influence has been tested is examined. The following table gives the protein, fat, and carbohydrate content of some of the cereals (Sherman [19]).

Table 18.

	Per cent. Carbohydrate.	*Per cent. Protein.*	*Per cent. Fat.*
Oatmeal	67·5	16·1	7·2
Rice	79·0	8·0	0·3
Wholemeal flour	71·9	13·8	1·9
White flour	76·4	7·9	1·4
Barley	77·8	8·5	1·1
Rye	78·7	6·8	0 9

It will be noticed that oatmeal, the worst offender so far as the production of rickets is concerned, contains the least carbohydrate (67·5 per cent.) while white flour and rice, the least rickets-producing, contain as much as 76·4 and 79·0 per cent. carbohydrate respectively. It would appear then quite definite that the cause of differences in the rickets-producing action of the various cereals must be sought along other lines than their carbohydrate content.

But while this is the case, it would be wrong to discount completely the part played by carbohydrates in problems of bone-calcification of growing animals. The more rapid growth which may result from additional carbohydrate in the diet will obviously call for a larger supply of those substances such as anti-rachitic vitamin and calcium essential for bone-calcification. Increasing the carbohydrate of the diet would be expected to bring about larger bones and, if the calcifying vitamin is deficient, relatively more defectively formed bones. Although, therefore, the part played by carbohydrate will not be considered further in this publication it is necessary to mention that its action in producing rickets or at least defective bone-calcification is probably of some importance. From the point of view of supplying material for growth purposes, the protein of cereal can probably be regarded also as an offender in a similar category to carbohydrate. It is clear, however, that the *differences* in rickets-producing effect of the cereals cannot be explained on the basis of their carbohydrate constituents.

(B) The Mineral Salt Content of Cereals.

Just as the clinician has often ascribed nutritional disorders in children following the ingestion of excessive cereal to its carbohydrate content, so the agriculturist has put the blame on its salt content. The ash of cereals contains so many mineral elements that it is obviously possible to formulate many hypotheses as to the manner in which variations in this group of substances might act. A few of these will be discussed in the light of the experimental results above described, and then further experiments dealing with the action of other cereal constituents will be dealt with.

(i) *The Absolute and Relative Amounts of Calcium and Phosphorus in Cereals.*

It is clear that the development of rickets must ultimately be brought into relationship with defects in the calcium and phosphorus metabolism. The diet of growing animals must contain sufficient calcium and phosphorus to provide that amount which is normally incorporated in perfect bones and other tissues. Otherwise, whatever the condition, the bones will either be imperfectly calcified or growth will be delayed.

The point of view is so obvious that it has received abundant consideration by those investigating the aetiology of rickets. Some indeed have thought deficient intake of calcium or of phosphorus or of both of these elements affords the explanation of the disease. Dibbelt [20], Heubner [21]. On the whole, however, this point of

view has not been generally accepted (Paton, Findlay, and Sharpe [22]). So far as human rickets is concerned I have discussed in an earlier publication [1 *d*] the question of calcium deficiency in the diet and shown how a diet deficient in anti-rachitic vitamin is often deficient in calcium, so that, although a deficient intake of calcium alone is not the cause of rickets, it is probable that the disease is often made worse by the added deficiency. Interest in this aspect of the subject has been revived on account of the large amount of work on experimental rickets carried out on rats in recent years. In the diet described by Sherman and Pappenheimer [16 *a*] for producing rachitic changes in rats there were several defects, one of which was a deficiency of phosphorus. An extension of this work by various investigators including McCollum and his co-workers resulted in the conclusion that, in order to produce rickets in rats, the diet must not only be deficient in the calcifying vitamin, but must also be deficient in phosphorus or calcium, or must contain these elements in unbalanced amounts. Some investigators working in England on the same subject (Korenchevsky [23 *a*, *b*], Goldblatt [24] &c.), have found it possible to produce in rats either rickets, or the related condition of defective calcification, osteoporosis, by diets deficient in the fat-soluble vitamin and without any particular abnormality in the calcium and phosphorus content of the diet. There is, however, but little doubt that a dietetic deficiency of calcium or phosphorus added to a vitamin deficiency assists the development of rickets in rats just as it does in puppies (*vide supra*, page 36). It may be added that the conditions of diet for the production of rickets in rats are generally so exacting as to be compatible with only small growth. In this respect it differs widely from the conditions associated with rickets in human beings or in puppies. It seemed possible that this aspect of the subject, namely, the intake of calcium and phosphorus, might throw some light on the variable rickets-producing effect of the different cereals.

The following table shows the amounts of calcium and phosphorus in some of the commoner cereals (Sherman [19]).

TABLE 19.

	Per cent. Calcium.	*Per cent. Phosphorus.*	*Ca.*	*P.*
Oatmeal	0·069	0·392	1	5·7
Rice	0·009	0·096	1	10·7
White flour	0·020	0·092	1	4·6
Whole meal flour	0·031	0·238	1	7·7
Barley	0·020	0·181	1	9·0
Rye flour	0·018	0·289	1	16·1
Wheat germ	0·071	1·050	1	14·8

These figures indicate that the relative rickets-producing effect of the cereals cannot be explained on the basis of absolute deficiency of calcium or of phosphorus or of both of these elements in the diet; for oatmeal, the most potent cereal in this respect, contains 0·069 per cent. calcium and 0·392 per cent. phosphorus as compared with white flour, the least potent cereal, which contains only 0·020 per cent. Ca. and 0·092 per cent. phosphorus. Here is an instance of a cereal which contains much more calcium and

phosphorus producing a greater defect in the deposition of calcium and phosphorus in bone than one containing less of these elements.

The same conclusion is reached when it is remembered that doubling the cereal in the diet, other things being constant, increases the rickets produced in experimental animals (Table 1). Doubling the oatmeal in the diet must increase the calcium and phosphorus ingested, and therefore the absolute amount of these elements in the diet cannot be of decisive importance.

It may be well to emphasize in this place, what I have previously stated elsewhere [1 *g*.], that these experiments dealing with the effect of cereals demonstrate the insignificance of phosphorus deficiency in the diet in the aetiology of rickets. Much stress has been laid by some American workers [11 and 15] on the question of phosphorus deficiency and its relation to rickets, and, even in seeking for the explanation of such conditions as starvation and sunlight on rickets, attention has been largely fixed on phosphorus metabolism. The results here described suggest that too much emphasis has been given to this aspect of the subject.

But, while the absolute amounts of calcium and phosphorus in the cereals do not explain their action in interfering with bone-calcification, it is necessary to consider the possibility of the relative amounts of these mineral elements being of importance in this respect. The point has been thought by some of the earlier observers to be of great significance. For instance Ingle [25] suggested that excess of phosphorus over calcium was responsible for the development of 'bran disease' or 'millers' horse rickets' developing in animals overfed with wheat offal. Weiske [26] found that the weight of the skeleton and the body weight of rabbits were increased when calcium carbonate was added to a diet of oats. The addition of sodium acid phosphate to oats resulted in a skeleton lighter in weight than when oats alone were eaten by rabbits. I have also suggested [1 *d*] that excess of phosphorus as compared with calcium might explain the rickets-producing effect of acid-caseinogen as compared with casein produced from milk by rennet. Acid-caseinogen contains no calcium but a fairly large amount of phosphorus, while casein contains some calcium as well as phosphorus. It seemed possible that acid-caseinogen was more rickets-producing because its phosphorus was oxidized to phosphoric-acid, and this in its excretion deprived the body of some of its calcium.

The importance of the *relative* amounts of calcium and phosphorus in the diet has also been pointed out and emphasized by McCollum, Simmonds, Shipley and Park [4 *b*, *c*]. As the result of their work on experimental rickets in rats they state that 'in so far as calcium and phosphorus are concerned the physiological relation in the diet between the two is of infinitely greater importance in increasing normal calcification than the absolute amount of the salts themselves'.

Sherman and Pappenheimer [16 *b*] have also stated that the quantitative relations of the inorganic ions are of greater importance than an absolute deficiency of any one of them.

Other evidence of the importance of a balanced calcium-phosphorus

intake is afforded by the experiments of Goldblatt [24 b], who found that increasing the calcium carbonate of a diet, previously used by Korenchevsky [23], so that the calcium-phosphorus ratio was altered from 1 : 0·80 to 1 : 0·20 transformed a non-rickets-producing into a definite rickets-producing diet.

From another direction also evidence is forthcoming that the balance of calcium and phosphorus intake is of importance in all problems of calcium and phosphorus metabolism. It has been shown for instance that an excess of calcium in the diet results in a larger amount of phosphorus being excreted in the faeces and less in the urine (Riesell [27], Lehmann [28], Strauss [29], and Herxheimer [30], &c. For a review of this subject see Forbes and Keith [31]). It appears that, although additional calcium in the diet slightly increases the amount absorbed, as is evident from the small increase in urinary calcium excreted (Rüdell [32]), the phosphorus of the food is retained to a large extent in the alimentary canal by the extra calcium and its absorption into the circulation inhibited (Telfer [33]). Similarly under some conditions an increase in the phosphorus of the food as by adding sodium acid phosphate results in an increase in calcium content of the urine and a diminution of faecal calcium (Telfer [33]). Thus it is clear that altering the balance between the calcium and phosphorus intake is capable of influencing greatly the absorption of both substances and of altering the supplies of each available to the body for assimilative purposes.

The facts suggest that in searching for an explanation of the action of cereals the calcium-phosphorus ratio of these substances must receive consideration. In Table 19, page 47, this ratio is set out. It might be expected that oatmeal and wheat germ, which have been shown to possess the most potent rickets-producing effect, would have a comparable calcium-phosphorus ratio but differing from that of white flour and rice, the least rickets-producing cereals. Contrary to this expectation, however, oatmeal and white flour have calcium-phosphorus ratios of the same order, i. e. 1 : 5·7 and 1 : 4·6 respectively, whereas rice and wheat germ have much higher ratios (1 : 10·7 and 1 : 14·8 respectively). In fact the complete discordance between the actual results and what would be expected on the basis of the hypothesis precludes further discussion on this point. In other words there is no support for the suggestion that the more intense rickets-producing effect of oatmeal under the experimental conditions described is due to a relatively large content of phosphorus as compared with calcium. It has also been shown that oatmeal contains actually much more calcium and phosphorus than the other cereals examined (Table 19) so that no solution of the problem seems obtainable in this direction.

While the action of cereals cannot be ascribed to their calcium-phosphorus content, there is no doubt but that their effect on bone-calcification can be greatly modified by altering the calcium and phosphorus of the diet, even when the anti-rachitic vitamin is very deficient. It has been shown above for instance that the addition of calcium carbonate to the rickets-producing diets inhibited

rachitic changes to a varying extent according to the amount of cereal eaten and the rate of growth of the puppy. Calcium phosphate also had a beneficial effect under the same conditions but to a less degree. In other experiments it was found that calcium acid phosphate in the diet also improved bone-calcification. The anti-rachitic effect of these salts is not, however, sufficiently potent under these conditions to lead to the belief that the calcium-phosphorus content of cereals holds the secret of their rickets-producing action.

(ii) *The Part Played by Other Mineral Constituents of Cereals and the Acid-Base Balance.*

While attention has been so far confined in this discussion to a consideration of the part played by the calcium and phosphorus in cereals, the other mineral elements must receive attention, for it has been urged that they also are of importance in this connexion. The suggestion has been made that foodstuffs tend to cause bone lesions in horses when they contain a relative excess of potassium to sodium. It has also been observed that, not only is the calcium in the various grains and their derivatives small, but that the ratios of magnesium to calcium and potassium to sodium are high.

From their experience of rickets in pigs, Elliot, Crichton, and Orr [35] considered that the main cause of this disease was abnormality in the salt content of the food. They found that the addition of calcium carbonate and sodium hydroxide to the diet of oatmeal, white bread, cod-muscle, swede turnip, produced great improvement in nutrition, and prevented rickets. The action of fat-soluble vitamin they considered of small importance in the aetiology of rickets in pigs. It may be said, however, that Zilva, Golding, and Drummond [34] have produced rickets in pigs on diets containing abundant calcium and phosphorus and in not unbalanced proportions when the fat-soluble vitamin was deficient.

The frequency with which the cause of rickets has been ascribed to an 'acidosis' suggests that the rickets-producing action of cereals might be related to the acid-base balance of their total mineral constituents. This point of view is also of interest in view of the beneficial action of the alkaline mixture (calcium carbonate and sodium hydroxide) used by Elliot, Crichton, and Orr in their work on rickets in pigs. On the other hand, in direct opposition to the 'acidosis' hypothesis, it has recently been stated by Jones, James, and Smith [36] that rickets can be *produced* in puppies when eating a complete diet, if its potential alkalinity is raised by the addition of potassium phosphate, and that this action of the diet can be prevented by adding to it hydrochloric acid.

It has also been seen above that calcium carbonate has a stronger anti-rachitic effect than calcium phosphate in puppies under certain conditions of diet, and it may be that this superiority is in part due to the acid-neutralizing effect of the carbonate. While these facts suggest the importance of the acid-base balance of the ashed mineral constituents of the diet so far

as the calcification of bones is concerned, there are other considerations which indicate that the problem is too complex to be settled on such a simple basis. The acidity or alkalinity of the ashed mineral constituents cannot supply definite information as to the balance of elements in the cereals themselves because of the presence of volatile and other organic substances which disappear on incineration, which must certainly play a part in the acid-base balance of any biological structure. Then, again, some of the inorganic elements are easily absorbed from the alimentary canal, and others only with difficulty, while physiological mechanisms of defence against acidity and alkalinity are manifold and include not only buffering mechanisms and the supply of neutralizing agents such as ammonia and carbon dioxide but a system of control of respiration, urinary secretion, and sweat gland activity, which ensures an almost constant neutrality of the blood and tissues, except for short periods and under great strain. The appreciation of this mechanism would not lead to the expectation that the total acid-base property of the mineral content of cereals will prove to be the controlling mechanism of the facts described.

Other evidence bearing on this question of acid-base balance and rickets includes that of McCollum, Simmonds, Shipley, and Park [4 d], who found that the substitution of calcium chloride for calcium carbonate or lactate, both of which raise the potential alkalinity of the diet, had no effect on the rickets-producing effect of their diets. They concluded that the reaction of their diets did not influence their rickets-producing power. Zucker, Johnson, and Barnett [37] come to the opposite conclusion, for in their experiments the substitution of calcium chloride for calcium lactate, or the addition of ammonium chloride converted a rickets-producing to a non-rickets-producing diet, while the addition of sodium carbonate acted in the opposite way.

There is obviously great discrepancy between the results and conclusions of the different investigators, and, for this reason alone, doubt arises as to whether the acid-base balance is of fundamental importance in this problem. It may be well, however, to examine the figures which are said to be a quantitative expression of the extent to which either the acid-forming or the base-forming elements in different foodstuffs predominate. It will be possible in this way to see whether there is any correlation between the acid-base ratio and the rickets-producing effect of cereals and other foodstuffs whose influence on the development of rickets is known. The figures in Table 20 were taken from Sherman's *Food Products* [19], and were obtained by calculating from the amounts of acid-forming elements the volume of normal acid which these elements could yield, and similarly the volume of normal alkali from the base-forming elements. The excess of acid or alkali is expressed in cubic centimetres of normal solution and is called the 'potential acidity' or 'potential alkalinity' of the substance. The figures given indicate the balance of acid-forming and base-forming elements contained in 100 caloric portions of each food:

TABLE 20.

Foodstuff.	*Acid.*	*Excess of Alkali.*
Oatmeal	3	—
Whole wheat flour	3·3	—
White flour	2·7	—
Rice	2·7	—
Barley	2·9	—
Milk (whole)	—	2·6
Milk (skimmed)	—	5·0
Beef (Can)	4·9	—
Cabbage	—	18·0
Carrots	—	24·0
Eggs	7·5	—

It will be seen that there is practically no difference in the acid-base numbers of the mineral content of the cereals. Oatmeal, for instance, which is much more potently rickets-producing than either whole meal or white flour has an acid number (3·0) which is lower than that of whole meal flour (3·3) and higher than that of white flour (2·7). There is, in fact, no support from these figures for the suggestion that the different effects of cereals on bone-calcification is to be found along these lines. As regards the acid-base figures given for a few other substances, some of which influence the development of rickets, it will be seen that they have no meaning so far as bone-calcification is concerned. Whole milk and eggs, both foodstuffs having potent anti-rachitic effects, have acid-base numbers of an opposite nature, that for whole milk being a 2·6 excess of alkali and for eggs a 7·5 excess of acid.

It must be concluded that the acid-base ratio of the mineral elements of foodstuffs is of but small relative importance in their action on bone-calcification, and certainly does not afford the explanation of the cereal effect.

C. THE ACTION OF SOME OTHER CEREAL CONSTITUENTS.

I have discussed above the rickets-producing effect of cereals in terms of known constituents of these substances which have been brought forward by different people as affecting the development of this disease, but important though some of them may be, they do not afford a satisfactory solution to the problem under discussion. It was, therefore, necessary, to extend the scope of the inquiry, and I propose now to deal with other constituents of cereals whose influence on bone-calcification has been studied in this work. These constituents are (i) nucleic acid (ii) the fatty substances.

(i) *Nucleic Acid.*

It is known that both oatmeal and wheat germ contain larger amounts of nucleic acid than are found in most other cereals. It is true that, so far as I am aware, there has never been reason to believe that the nucleic acids are toxic, but influences sufficient to explain the effect of cereals on bone-formation are of a low order of toxicity, and are so insidious and slow in producing their results that they might easily be missed.

In the experiments made to test this suggestion, a commercial

preparation of nucleic acid made from yeast was used. No doubt it would have been more appropriate had the nucleic acid used in the experiments been prepared either from oatmeal or wheat germ. The evidence, however, shows that yeast nucleic acid is identical with triticonucleic acid obtained from wheat embryo. Triticonucleic acid, first prepared from wheat germ by Osborne and Harris [38], was also found by them to give the same end-products of hydrolysis as yeast nucleic acid. These observations were confirmed by Levene and La Forge [39], when they found that acid-hydrolysis of wheat germ nucleic acid at 175° C., under pressure with ammonia, yielded three of the nucleosides of yeast nucleic acid, viz. guanosine, adenosine, and cytidine, and also that the pentose of the germ product was *d*-ribose as in the case of yeast nucleic acid.

Seven experiments were made to test the action of nucleic acid on rickets. To diets made up of separated milk, bread, meat, olive oil, and orange juice, the commercial preparation of nucleic acid (1 to 2 gms. daily) was added, but no evidence was obtained to show that this substance had a definite rickets-producing effect. In other experiments yeast itself as a source of nucleic acid was added in fairly large quantities to the diets. In an earlier publication I pointed out that yeast eaten by puppies in quantities of 5 to 10 gms. daily did not have any special action on rickets. In these later experiments it was found that even 40 gms. of yeast daily had no obvious effect in hastening rickets. In experiments using yeast it is, of course, essential to kill the cells by heat before giving it to the animals. I have obtained no evidence up to the present time that nucleic acid is the substance in cereals responsible for the rickets-producing effect of these foodstuffs.

(ii) *Fatty Substances.*

Another chemical constituent of a distinctive nature present in appreciable quantities in oatmeal and wheat germ is fat. It will be seen from the figures on p. 45 how large is the fat content of oatmeal (7·2 per cent.) as compared with the amount in white flour (1·4 per cent) and rice (0·3 per cent.). Although it is usual to ascribe many of the supposed advantages of oatmeal over other cereals to its relatively high fat content, it seemed desirable, especially in view of the failure of investigations along other lines, to see whether this constituent had any detrimental action so far as bones are concerned.

In the case of wheat germ there was already evidence from the experimental work of McCollum, Simmonds, and Pitz [40] that something in the germ that could be partially extracted by ether was capable of exerting a toxic action. They pointed out that, although wheat germ contained protein of good biological value, some vitamin A and abundant vitamin B, yet it interfered with the growth of their rats on synthetic diets when it formed too large a portion of their diets. Its action could be overcome in some degree by an addition to the diet of 5 per cent. casein and also by increasing the butter from 2 per cent. to 5 per cent. of the food eaten. They could not decide whether the toxic product of germ was the

fatty substance or was only associated with the fatty substance. An extension of this work is to be seen in the observations of Hart, Miller, and McCollum [41] on the effect of rations containing a large amount of wheat on pigs. They produced malnutrition and degenerative changes in the nerves similar to those found in beri-beri by diets containing excessive cereals, but these authors point out that the pathological changes in the spinal cord were due to the inherent toxicity of the grain and not, as in beri-beri, to a deficiency of vitamin B. In these feeding experiments on swine the toxic effect of the wheat could be greatly mitigated by additions to the diet of alfalfa and commercial meat scraps. It was suggested that the beneficial effects of these additions were due to their vitamin A content, the salt mixture, better proteins, and a liberal supply of calcium phosphate.

Osborne and Mendel [3] were unable to confirm the results of McCollum, Simmonds, and Pitz [40] as to the toxic action of wheat germ on growing rats, but the diets used by Osborne and Mendel were different from those used by McCollum, Simmonds, and Pitz. They contained, for instance, 9 per cent. butter fat, as against 2 to 5 per cent. present in McCollum's diets. This is an important point, because, both according to the results of McCollum and his colleagues and the experiments described in the present publication, the detrimental effects of wheat germ can be easily antagonized by other constituents of a normal diet. There was no reason, however, to believe that the substance in wheat germ which interfered with the growth of rats in the experiments of McCollum, Simmonds, and Pitz had anything to do with the rickets-producing effect of cereals mentioned above.

Before describing some of the experiments made to test the question as to the influence of the fat content of oatmeal and wheat germ on bone-calcification, some points, including the mode of preparation of these fatty substances, will be mentioned. Two methods of preparation have been used. (1) The extraction of oatmeal and wheat germ with fat-solvents such as acetone and ether. In the case of oatmeal it is necessary to dry it before extraction with ether. (2) Saponification by heating with alcoholic caustic soda and solution of the fatty acids into petrol ether. 200 gms. of cereal, 44 gms. of caustic soda in 40 c.cms. of water and 500 c.cms. methylated spirit were heated in a flask with reflux condenser for $\frac{1}{2}$ to 1 hour. When the unsaponifiable substances were required, the mixture was twice extracted with petrol ether while alkaline. After this it was acidified and the saponifiable substances extracted twice with fresh petrol ether. Sometimes the saponifiable and unsaponifiable substances were obtained together by acidifying directly after heating with soda and then extracting with petrol ether.

The following figures show the amount of the different products obtained by the above-described methods.

Oatmeal.

On drying loses 9 per cent. of its weight.

Dried oatmeal, on saponification with soda, gives 0·15 per cent.

unsaponifiable substances soluble in petrol ether and 7·5 to 8 per cent. saponifiable substances.

Wheat germ.

The germ used in these experiments is known as 'commercial wheat germ', and is a mixture of germ and bran. Different samples have varied as to the relative amount of these components, so that the yield of fatty substances has also varied. The less bran present in the specimen the greater is the percentage yield of fatty substance. In the preparation of the products the germ has not been dried before extraction.

Commercial wheat germ, on ether extraction in a Sohxlet apparatus, has given 6 to 7 per cent., while acetone extract has given 7 to 8 per cent. yield.

Wheat germ, after saponification with soda, has given about 0·5 per cent. unsaponifiable material and 9·3 to 9·5 per cent. of saponifiable substance.

All the earlier experiments made to test the action of the fatty constituents of oatmeal and wheat germ were carried out with their acetone- and ether-soluble substances. The results were neither consistent nor easy to interpret. One difficulty was that in nearly all cases the experimental animals developed severe rickets, and it was not easy therefore to determine the relative intensity of the pathological condition. Another cause of difficulty was that, although a rickets-producing substance appeared in some cases to be extracted from wheat germ and oatmeal by ether and acetone, the removal was only partial. In the case of oatmeal most of the active substance remained after extraction.

Many other experiments were carried out using different methods of treatment, such as extraction with alkaline and acid alcohol solutions. It has not been possible by these methods to come to any certain conclusion as to the nature and properties of a substance in oatmeal which has potent rickets-producing properties.

In view of the uncertainty of the results obtained as to the relation of those fatty substances which could be easily extracted from oatmeal and wheat germ to the toxic effect of these foodstuffs, it seemed desirable to prepare the total saponifiable and unsaponifiable substances soluble in a fat-solvent and to test their effect. These substances were therefore prepared as described above, and their action tested in a few experiments. This part of the investigation is being actively pursued, and the present results must be regarded as of a preliminary nature only.

The following series of experiments is a record of one investigation carried out on these substances.

Effect of Saponifiable and Unsaponifiable Substances of Oatmeal and Wheat Germ.

Experiments 802 *to* 807.

Age at beginning of experiment: 7 weeks.

Diet eaten by all included separated milk powder 20–30 gms., meat 10 gms., white flour 30 to 70 gms., orange juice 3 c.cms., yeast 3–5 gms., olive oil 10 c.cms.

In addition, 802 received unsaponifiable substances of germ.
803 „ saponifiable „ „ „
804 „ unsaponifiable „ „ oatmeal.
805 „ saponifiable „ „ „
807 „ wheat germ as substitute for 40 per cent. of wheat flour.

806 was the control puppy and ate the diet without any addition.

The amount of saponifiable and unsaponifiable substances added were based on the diet of the control animal 806, which received white flour only as its cereal. For instance, the puppies 804 and 805 received the amount of additional products which were extracted from oatmeal equivalent in amount to the white flour eaten by 806. 802 and 803 received in their diets daily just as much unsaponifiable and saponifiable substances respectively as was contained in the germ eaten by 807. Suppose 806 on a particular day received 100 gms. of white flour, then 807 received 60 gms. white flour and 40 gms. commercial wheat germ. On that day 802 and 803 would each receive 100 gms. of white flour, but 802 would also get the unsaponifiable substances made from 40 gms. of germ, i. e. about 0·2 gms. daily, and 803 the saponifiable substances from the same amount of germ, i. e. about 4 gms. 804 and 805 would also receive 100 gms. of white flour, together with amount of unsaponifiable and saponifiable products that can be obtained from 100 gms. of oatmeal, i. e. 0·15 gm. and 7 gms. respectively.

TABLE 21.

No. of Expt.	*Diet. Variable.*	*Duration of Expt. Weeks.*	*Weight.*			*CaO in Femur Shaft.*			*Histology.*
			Initial. gms.	*Final. gms.*	*Max gms.*	*Percentage.*		*Amount. gms.*	
						Dry.	*Fresh.*		
802	Unsaponifiable substances in germ	12½	1015	3520	3520	20·1	13·0	0·75	Practically normal
803	Saponifiable substances in germ	12½	1165	3000	3080 after 9½ weeks	15·2	9·3	0·45	Slight rickets, more osteoid tissue than 806 and 802
804	Unsaponifiable substances in oatmeal	12½	1085	3800	3800	19·6	13·6	0·67	Practically normal
805	Saponifiable substances in oatmeal	12½	1220	2300	2980 after 6½ weeks	17·0	6·8	0·33	Bad rickets
806	Control	13	1165	3600	3600	17·7	12·0	0·61	Slight rickets
807	Germ	13	1325	3730	3730	16·3	10·7	0·70	Rickets

Figs. 85, 86, 87, 88, 89, and 90 are radiographs of these animals taken after 12½ weeks of the experimental diets. It will be seen that the bones of 802 (Fig. 85), 803 (Fig. 86), and 806 (Fig. 89), are normal, 807 (Fig. 90) shows some rickets, while 805 (Fig. 88) has very bad rickets. Histological examination of the bones is in general agreement with the radiographic results. 803 has rather

more osteoid tissue than 802 and 806, but not so much as 807 and 805. Figs. 91, 92, and 93 are photomicrographs of the costo-chondral junction of 806 (control) (Fig. 91), 805 (saponifiable oatmeal) (Fig. 92) and 807 (wheat germ) (Fig. 93). They show clearly the amount of calcification in the bones of the different animals as well as the disorganization of growth in 805 and 807.

As for the calcium results, it is worthy of comment that the amount of calcium oxide in the femur shaft of 803 is lower and in 807 higher than would be expected from radiographic and histological examination.

The weight curves of this series of experiments are given in Fig. 94. For about 50 days the experiment was satisfactory, and the animals increased in weight at a similar pace. After this, however, 805 (saponifiable substances of oatmeal) went off its food and lost weight progressively till the end of the period. 803 (saponifiable substances of wheat germ) also ceased about this time to finish off its ration, and its rate of increase in weight became smaller than that of the other animals of the series. In my experience this loss of appetite in puppies whose diet includes the saponifiable substances, more especially of oatmeal, is common, but the explanation is not known at present. Post-mortem examination did not reveal that the oatmeal preparation was a gastro-intestinal irritant.

The outstanding result of this series of experiments is that the substances in oatmeal which are saponifiable have produced severe rickets (805). The unsaponifiable substances in oatmeal and germ have certainly not had a rickets-producing effect (804 and 802), and, in fact, there is some suggestion in the evidence that they have had an anti-rachitic action. The animal eating commercial wheat germ (40 per cent. of cereal) has developed fairly bad rickets (807) as compared with the control animal (806) with only slight rickets.

The addition of the fatty acids of wheat germ (803) did not have the gross effect on bone-formation such as appeared in 805 (saponifiable substances of oatmeal), but the relatively low percentage of calcium oxide present in the femur shaft indicates some interference with the deposition of calcium salts. It is necessary to remember, however, that 803 only received on an average 2 to 3 gms. of fatty acid from germ as compared with 5 gms. of these substances from oatmeal added to the diet of 805. The addition of wheat germ to the extent used in these experiments, i. e. up to 40 per cent. of the total cereal, has never intensified the rachitic condition of puppies so severely as oatmeal (100 per cent. of cereal), so it is possible that the interference with bone-calcification evident in 803 may be an indication that the fatty acid derivatives from germ contain a small amount of the substance responsible for the action of 'commercial wheat germ'.

The Action of Combined Saponifiable and Unsaponifiable Substances in Oatmeal and Wheat Germ.

In the previous experiments the action on calcification of the saponifiable and unsaponifiable substances of oatmeal and commercial

wheat germ were tested separately. In the case of the oatmeal products the saponifiable substances resulted in intensification of the rachitic changes, whereas there was a slight indication that the unsaponifiable substances had an anti-rachitic effect. As regards the latter point, the fact that the control animal was only slightly rachitic at the end of the experiment did not allow any more definite deduction as to their action. In the following experiments the saponifiable and unsaponifiable substances were added together. The oatmeal and wheat germ were boiled up with alcoholic soda; the mixtures were then acidified and extracted with petrol ether, and the soluble portions used in the following experiments.

Experiments 847 *and* 848.

Age at beginning of experiment: 7 weeks.

General daily diet eaten by the puppies consisted of separated milk powder 20 gms., meat 10 gms., yeast 5 gms., orange juice 3 c.cms., sodium chloride 1–3 gms., white flour 50 to 90 gms.

847 ate this diet only.

848 received, in addition, the saponifiable and unsaponifiable substances present in an amount of oatmeal equal to the white flour eaten at any period. The total amount of oatmeal product eaten by 848 varied, increasing as the amount of white flour eaten increased. During most of the experimental period about 4·5 gms., representing the amount of saponifiable and unsaponifiable substances in 50 to 70 gms. of oatmeal, was added to the diet of 848.

It will be seen in the radiographs of 847 and 848 (Figs. 95 and 96), taken after 13 weeks of the experimental diet, that 848 had developed rickets, but that 847 (control) had only slight rickets. The saponifiable and unsaponifiable substances of oatmeal acting together had, therefore, exerted a rickets-producing effect, but this appeared to be smaller than the effect of the saponifiable substances of oatmeal acting alone. Apparently either the unsaponifiable substances had exerted an anti-rachitic action, and thereby neutralized some of the rickets-producing effect of the saponifiable substances, or that part of the substance responsible for the result in Exp. 805 Fig. 88 was either not extracted or was destroyed at some unknown stage in the preparation of the material used in Exp. 848. Which of these suggestions accounts for the above results can only be decided by further experimental work. It might be expected that the unsaponifiable substances in oatmeal would be anti-rachitic, for there is no doubt but that grains such as oats contain some fat-soluble vitamin, and it is generally recognized that this vitamin or these vitamins are associated with the unsaponifiable part of the fat.

These experiments dealing with the fat of cereals suggest that some of the rickets-producing effect of oatmeal will ultimately be traced to its fatty acid content. The product obtained by the saponification method has a loathsome smell and is toxic to animals, and this makes it difficult to carry through successfully experiments made to test its effect on bone-formation. In several other series of experiments the animals taking it have become ill and refused to eat their food after about a month of the diet, and

this period is too short to allow clear deduction as to the specific effect of the substance on bone-calcification.

The question arises as to whether the rickets-producing effect of the saponifiable substances in oatmeal evident in Exp. 805 (Table 21) is a general fatty acid action or whether it is due to the specific fatty acids of oatmeal or to some substance associated with the fatty acids.

Most of the feeding experiments with fatty acids do not suggest that ordinary acids in general have a rickets-producing effect. For instance, Lander and Fagan [42] fed pigs on diets containing the fatty acids of coco-nut oil, and Burns and Sharpe [43] fed men on diets containing the fatty acids of hardened whale oil. Their results indicated that within limits fatty acids are dealt with in a similar way to fats themselves, and did not lead to the belief that the addition to the diet of small quantities of ordinary fatty acid either deprives the animal of much calcium or is responsible for the chronic ill health and rickets such as appears to be brought about by the fatty acids of oatmeal. On the other hand, it is possible that fatty acids as such might have a noxious influence under the specific experimental conditions of this investigation, and more especially when the intake of fat-soluble vitamin is very small. If this is not the case there remains the possibility that the oatmeal fatty acids contain some specific rickets-producing member.

It may be asked whether there was any evidence in these experiments of the toxic factor in wheat which, according to Hart, Miller, and McCollum [41], produces malnutrition and degenerative changes in the nerves of pigs. The effects of the experimental diets are often so widespread, and include so many abnormalities in different organs of the body, that it is difficult to say definitely that wheat germ had any specific action in producing symptoms. In particular, most cases of severe rickets become weak and often paralysed, especially in the hind legs. In some experiments, however, it seemed that commercial wheat germ had a peculiar effect of its own. For instance, both in 803 (saponifiable substances of wheat germ) and 807 (wheat germ) the animals developed a peculiar nervous condition, the most obvious feature of which was the inability to walk or run straight. The animals seemed to lose their sense of balance and their heads moved from side to side when they attempted to walk, and finally they fell over. At a later period they were unable to stand, partly because of their loss of balance and partly because of muscular weakness. These conditions were not obviously related to the development of rickets because, as explained above, 803 was practically free from rickets and yet was the more affected of the two dogs. The other animals of this litter, even 805 (saponifiable substances oatmeal), which developed severe rickets, did not show any of the symptoms of unbalance seen in the germ-eating dogs, although 805 developed great muscular weakness. In five other cases where germ or ether or acetone extract of germ has been included in the diet of puppies notes were made to the effect that the animals show lack of balancing power. There are several cases, however, where there was no such development under these or

closely allied dietetic conditions. Evidence for the presence of a constituent of germ partially removed by ether extraction, and capable of acting on the nervous system and especially of bringing about loss of balance, is fairly strong in these experiments, but is not conclusive. If this substance is also present in other cereals, it must be in smaller amounts than in wheat germ. On the other hand, the substance in oatmeal which interferes so strongly with bone-calcification is present in smaller amounts in wheat germ. It would be interesting if it should prove that the different effects of toxic action produced by oatmeal and wheat germ, when forming large parts of diets deficient in anti-rachitic vitamin, should depend on different substances both of which are associated with the fatty acid fraction.

The rickets-producing effect of cereals has now been considered from different points of view, and, although the experimental evidence described above is of a preliminary nature, it suggests that two factors, at least, are responsible for the effect. One of these is the part of the grain which allows increased growth. The second is probably some chemical constituent which interferes with the calcification of the growing bone.

As regards the first of these, although to the carbohydrate content of cereals must be apportioned some part of the responsibility for growth, the protein present in them must also be considered as of importance in this connexion. If cereals contained some factors which hastened calcification processes in bone, in addition to those substances which are incorporated in the growing organism, normal bone tissue might still be laid down under their influence. Unfortunately, not only do they not appear to contain a substance of this nature of any potency, but, in the one cereal more closely examined, namely oatmeal, there is evidence of a chemical body which actively interferes with the mechanism for laying down calcium salts in the bones. A substance with this property may also be present in wheat germ and in other cereals, but the evidence of this is at present smaller, and in any case its activity is much less apparent in the amounts used than that of the same factor in oatmeal.

It is too early to speculate either as to the chemical composition of the active substance or as to its mode of action. Even if the results described above showing the interfering effect of the fatty acid content of oatmeal on bone-calcification should prove to be specific and not simply due to their fatty acid constituents as such, they do not appear to account for the whole oatmeal action. On the other hand, some part of the fatty acid influence may have disappeared as the result of the drastic chemical treatment which was used in their preparation. As regards the mode of action of the substance, its influence is so diametrically opposed to that of the anti-rachitic vitamin that the possibility of it being an anti-vitamin suggests itself. This, however, is pure speculation. Not only does it interfere with the laying down of calcium in bones and also, as shown by M. Mellanby [44 (*a*)], in teeth, and thereby act in opposition to the anti-rachitic vitamin, but just as the vitamin and ultra-violet radiation assist each other, and are to some extent

interchangeable, so are the cereal influence and the radiations opposed in their action. The same kind of antagonism seems to extend to other organs, and more especially to the formation and activity of the voluntary muscle (Clifford, Surie and E. Mellanby, Communication to Physiological Society 1923, Nov.).

It would be interesting if it should prove that there is a specific substance interfering with calcification related in some way to the fatty content of oatmeal, especially in view of the fact that the anti-rachitic factor is classed as a fat-soluble vitamin. However, it has been pointed out above that, unlike fat-soluble vitamin, which is said to be associated more closely with the unsaponifiable fraction, the rickets-producing substance in oatmeal is absent from this fraction.

It is clear that just as the subject of nutrition has had to be revised in the light of recent work on vitamins, so also the study of the action of cereals described above, bringing evidence of another substance and possibly a class of substances, hitherto unknown, which play an important part in the animal economy, must also receive due consideration in nutritional problems. It would appear that even the simplest problems of nutrition are far more complicated than has been previously imagined. For instance, the question of mineral salt balance has generally been considered as a self-contained subject. But even in the case of calcium it is now evident that calcium metabolism is controlled apart from salt balance, by a series of dietetic factors including, among others, the anti-rachitic vitamin, and the substance in oatmeal and probably other cereals. In addition, the environment also influences the same metabolic process by means of the ultra-violet radiations to which the organism and, in some cases, the foodstuff is exposed. It would appear useless to attempt to explain the deposition of calcium phosphate in bone in terms of mineral salt metabolism until more is known about the dietetic and environmental factors which control so powerfully the distribution of these elements and the changes undergone by them in the body.

Evidence is accumulating which suggests that the subject of toxic products in cereals may be one of great importance. In the light of this work demonstrating the interfering action of cereals on bone-calcification, and the experiments in which beri-beri-like symptoms were produced by Hart, Miller, and McCollum [41] in pigs by feeding with whole grain, it appears not improbable that the symptoms and signs of pellagra found among maize-eating populations may be due, partly at least, to some toxic agent in the maize. It is possible that this aspect of nutrition may be developed and its importance emphasized when further attention is given to it. It is an interesting fact that toxic conditions which can be produced or are associated with different cereals only become evident under certain conditions, and that other dietetic factors when eaten in sufficient amounts are able to antagonize and prevent the detrimental results. The recognition of 'deficiency diseases' and their prevention in some cases by vitamins was responsible for the introduction into dietetic phraseology of the words 'protective foodstuffs'. Foodstuffs rich in anti-rachitic vitamin are certainly 'protective' in

that they improve the general nutrition and prevent the development of certain pathological conditions, but the evidence given above demonstrates that one important and unsuspected way in which they protect is by preventing the toxic action of certain constituents of other foodstuffs.

VII. SUMMARY OF RESULTS.

In this report, which is a continuation of the investigation published in No. 61 of the Special Report Series (Medical Research Council), I have described experiments made with the object of developing another aspect of the problem of rickets. Evidence has been given which shows that some cereals and cereal products have a powerful interfering influence on bone-calcification, varying with the amount and type of cereal eaten and with the general make-up of the diet.

When the diet is deficient in anti-rachitic vitamin, increasing the amount of cereal brings about worse-formed bones if other factors of diet and environment remain constant. Qualitative differences between the effects of the different cereals are also great. Among those substances tested oatmeal has pre-eminently the worst influence on bone-formation, and after that come maize and barley, rice, and wheaten flour, the last having the least detrimental effect. There is but little difference between the rickets-producing effect of rice and flour. The germ of wheaten flour, when added to the diet in sufficient quantities, also interferes with bone-calcification.

The action of cereals on bone-calcification is antagonized to varying degrees by foodstuffs containing the anti-rachitic vitamin. As might be expected, cod-liver oil is much the most effective substance in this respect, and is capable, even in small quantities, of preventing the cereal action. Whole milk, in fairly large quantities, and egg yolk are also potent antagonists to the cereal effect, but butter is of comparatively small value unless it is accompanied by a fairly high calcium intake. Both calcium carbonate and calcium phosphate work well in conjunction with the vitamin of butter-fat. The anti-rachitic effect of separated milk, either when working in conjunction with butter-fat or in an independent way, is more potent than can be accounted for by its calcium content alone.

Even when the diet is greatly deficient in anti-rachitic vitamin, increasing its calcium content, either by adding calcium carbonate, calcium phosphate, or calcium acid phosphate, hinders to some extent the development of rickets. Under these circumstances calcium carbonate is more effective than calcium phosphate.

Evidence is also given to show that the detrimental effects of cereals, which become prominent in diets deficient in anti-rachitic vitamin, can often be antagonized to some extent either by exposing the animal eating them to some source of ultra-violet radiations or by exposing the cereal itself to the same radiations.

As regards the method whereby cereals bring about this rickets-producing effect two different kinds of action appear to be at work. In the first place that part of the cereal which is actually in-

corporated in the growing organism and leads to its growth is probably partially responsible. In this way the carbohydrate and the protein would be involved. In the second place there is present in the one cereal tested, namely oatmeal, a chemical grouping which, after digestion and absorption of the grain, is capable of interfering with bone-calcification. After many attempts had been made to explain this action in terms of known constituents of cereals and to find out the nature of the causative agent or agents, some evidence has finally been obtained which suggests that a substance in oatmeal which interferes with the laying down of calcium in bones is associated with the fatty acids. This substance can be obtained by saponification of oatmeal with soda and extracting the acidified mixture with petrol ether.

Further work is necessary to establish the nature and properties of this cereal constituent and also to determine its mode of action. In the meantime evidence of the presence of such a substance helps towards the solution of a difficult problem, namely, that oatmeal, which contains more calcium and more phosphorus than other cereals tested, should, in the absence of sufficient anti-rachitic vitamin, result in the deposition of the smallest amount of these elements in growing bones. White flour contains much less calcium and phosphorus than oatmeal, but results in better bone-formation, when the anti-rachitic vitamin intake is deficient, because it contains much less of the substance or substances interfering with ossification.

Since the anti-rachitic vitamin supplies in the food and exposure of the animal or of the food eaten to ultra-violet radiations tend to conserve ingested calcium and phosphorus for the use of the growing organism, and since the cereals work in the opposite direction, it is evident that the amount of calcium and phosphorus in the food is of but secondary importance in the control of the deposition of these elements in growing bone, although, of course, there must be a sufficiency of these salts for the formation of perfect bones. In view of the evidence of interaction and balance among food constituents provided by this investigation, the value of the expression 'optimum calcium content of a diet' so commonly used in dietetic descriptions and discussions nowadays must be doubted. The optimum varies every time the other elements of the diet are changed.

There are one or two practical points of dietetics which arise out of these experimental results. The challenge, for instance, immediately may be made, if oatmeal is so detrimental to bone-formation, how is it that fine races of men have been reared on diets of which this cereal forms a large part? If these results apply to man, as they almost certainly do in the case of the teeth and probably therefore, as regards other tissues (M. Mellanby, 44 *b*), then it is highly probable that the diet of these people also included much of foods rich in anti-rachitic vitamin, as, for example, milk, eggs, fish of the fatty variety, including herring, salmon, mackerel, &c. In tropical countries where cereals such as rice, maize, millet, form a large part of the diet, the sunlight is no doubt also an important factor in antagonizing their detrimental influence. Whether these

answers are entirely satisfactory or not only further investigation can determine, but the present work does show that perfect bone-formation can be obtained even when large quantities of oatmeal are eaten if the rest of the diet be adequate. On the other hand the worst cases of malnutrition seen in human beings can be easily reproduced in animals by feeding them on foodstuffs which bulk largely in the national dietary along the lines described in this investigation. Apart from extreme malnutrition, however, it would appear not improbable that in this country, where the average diet is either deficient in or contains a border-line quantity of anti-rachitic vitamin and calcium, and where sunshine is negligible, the ingestion of oatmeal during pregnancy and lactation of women, and in growing children, does much harm.

A second challenge centres round the controversy of whole meal bread and the nutritive properties of wheat embryo. A discussion of this question cannot be included here, for it would necessitate the study not only of the protein and vitamin content of wheat embryo, including vitamins A and B and the newly-discovered vitamin concerned with fertility and said by Evans and Bishop [47] to be abundant in this part of the grain, but also of the toxic product or products present in it. It may be well, however, to point out that in the experiments described above, where evidence of the rickets-producing action of wheat germ was given, as much as 20 to 40 per cent. of the cereal eaten was in the form of 'commercial wheat germ' and that the diets were deficient in anti-rachitic vitamin. Some of this product was bran, but, even so, the amount of germ eaten by the animals must have been much higher than 1·5 per cent. present in whole wheat. From the point of view of rickets and its allied problems the question of whole meal versus white bread is probably of but little practical importance. On the other hand the feeding of animals with large quantities of cereal offal, including germ, certainly can and does lead to detrimental results, which can, however, be easily avoided by adding other foodstuffs of the right nature. To those who ascribe special virtues to various grains because of their calcium and phosphorus content, the experimental results above described ought to bring a special message of caution.

Miss Dorothea Selby, B.Sc., and Miss Ella Surie, B.Sc., have had control of the actual feeding experiments at different times. I wish to express my indebtedness to both of them for carrying out what is a most exacting labour.

The Field Laboratory, where the foregoing experimental work was done, was specially erected by the University of Sheffield for this purpose. The expenses of this investigation, as of the earlier work carried out by me on rickets, were provided by the Medical Research Council. I wish to express my thanks to both of these bodies.

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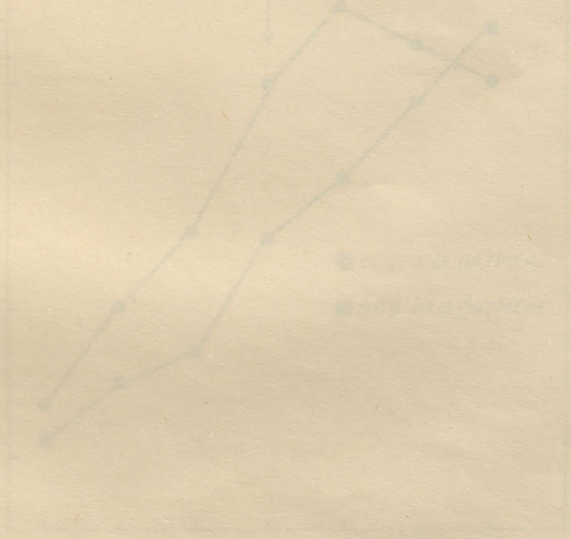
ILLUSTRATIONS

FIGURES 1–102



FIGS. 1 and 2. Radiographs after 9 weeks of diet.

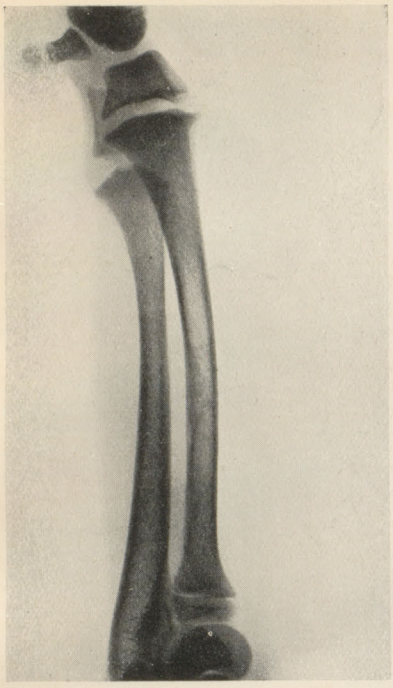


FIG. 1. Exp. 502. Diet included x grms. of oatmeal.

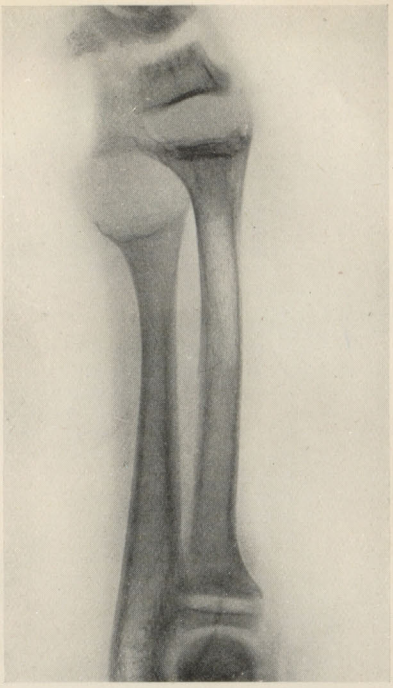


Fig. 2. Exp. 509. Diet included 2 x grms. of oatmeal.

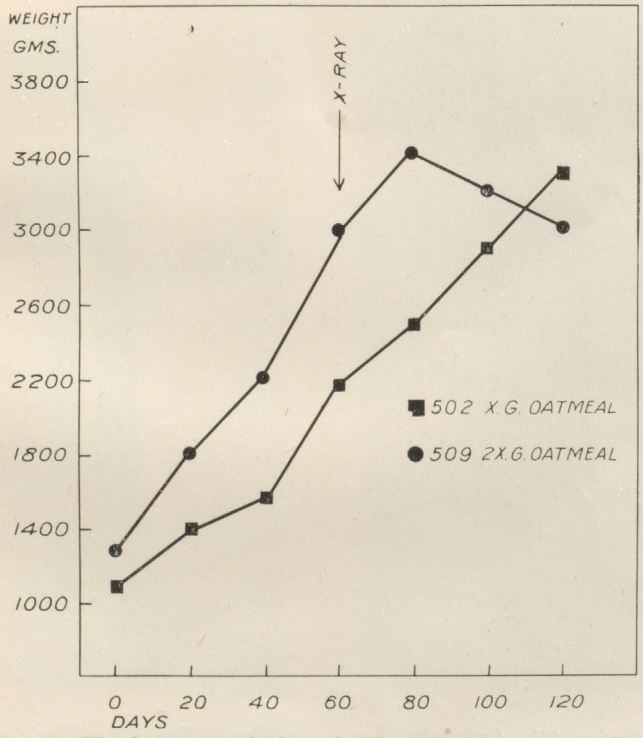


FIG. 3. Weight curves of 502 and 509. 509 grew more rapidly in the earlier weeks but began to lose weight after 12 weeks of diet owing to severe rickets and consequent loss of appetite.

FIGS. 4, 6, and 7. Radiographs after 14 weeks of diet. (Fig. 5. After 12 weeks.)

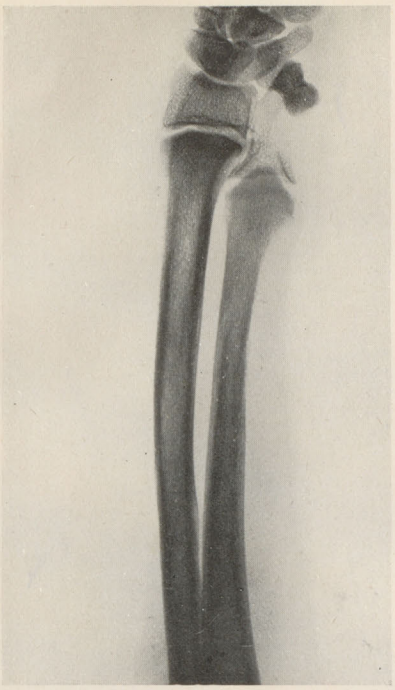


FIG. 4. Exp. 418. White flour.

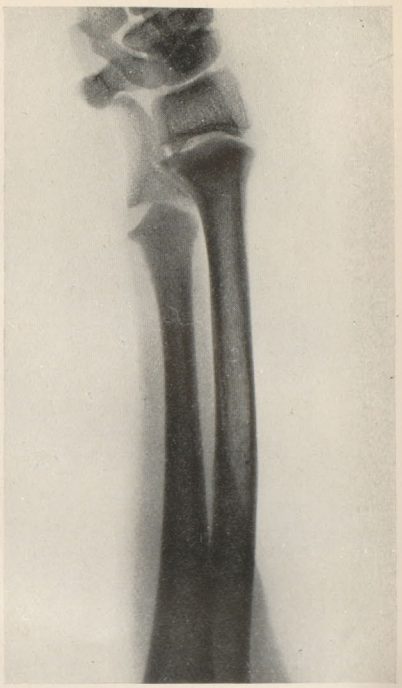


FIG. 5. Exp. 419. Rice (unpolished).

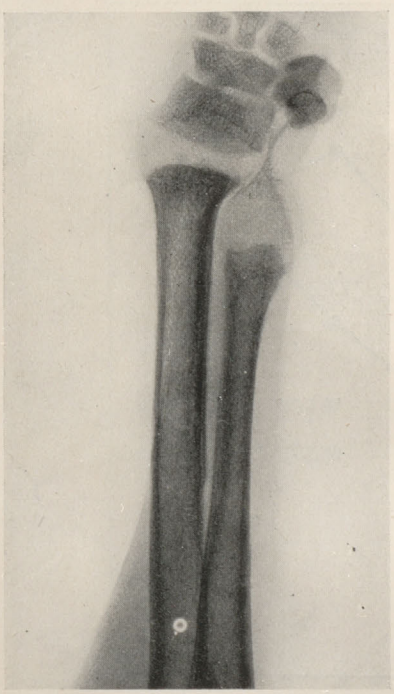


FIG. 6. Exp. 421. Oatmeal.

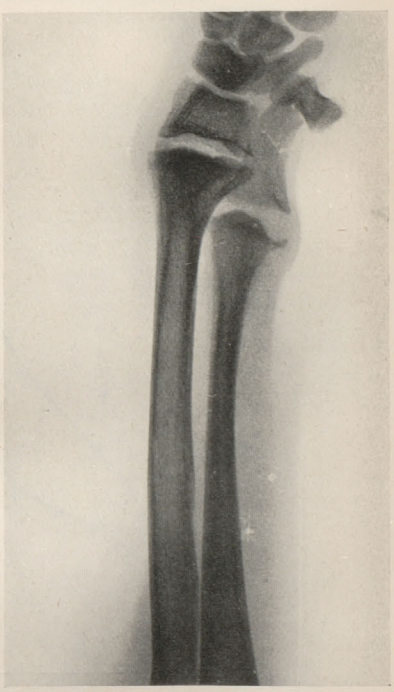


FIG. 7. Exp. 422. Whole meal flour.

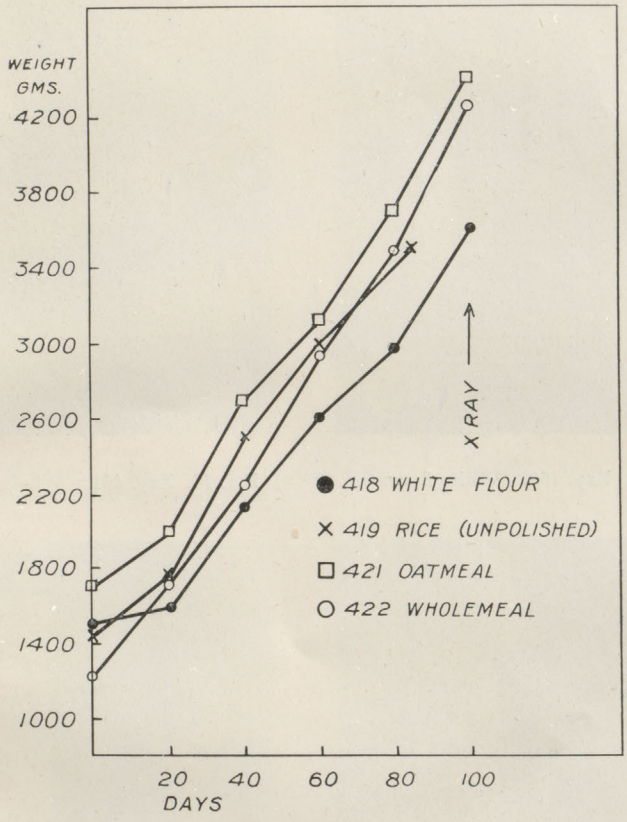


FIG. 8. Weight curves of 418, 419, 421, and 422.

Figs. 9-14. Radiographs taken after 12 weeks of diet.

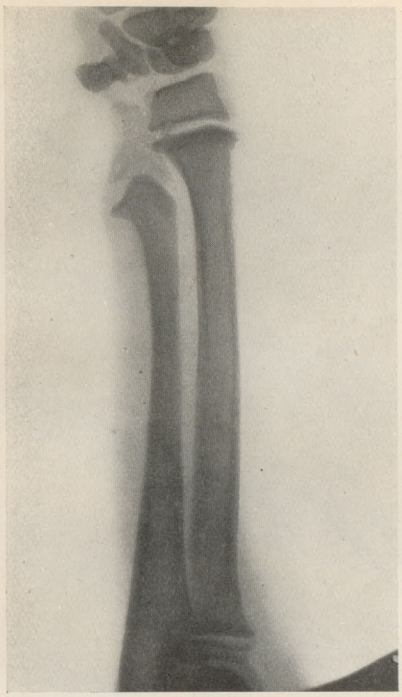


Fig. 9. Exp. 460. White flour.

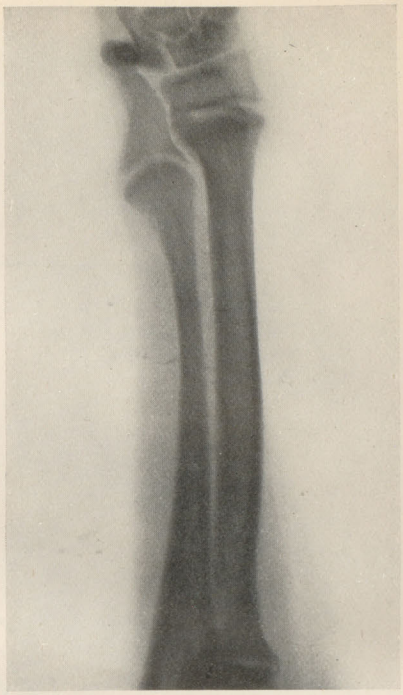


Fig. 10. Exp. 461. Whole meal flour.

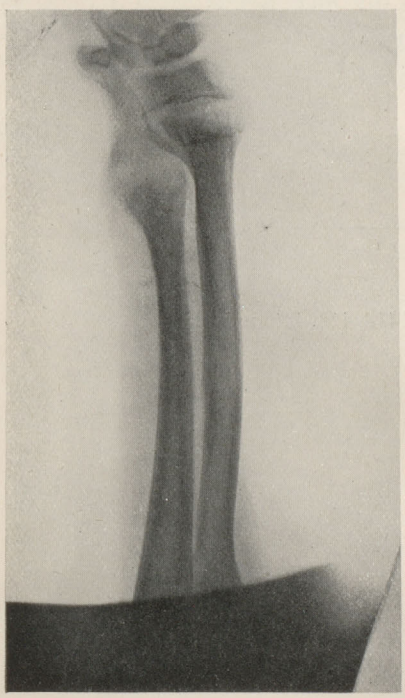


Fig. 11. Exp. 462. Oatmeal.

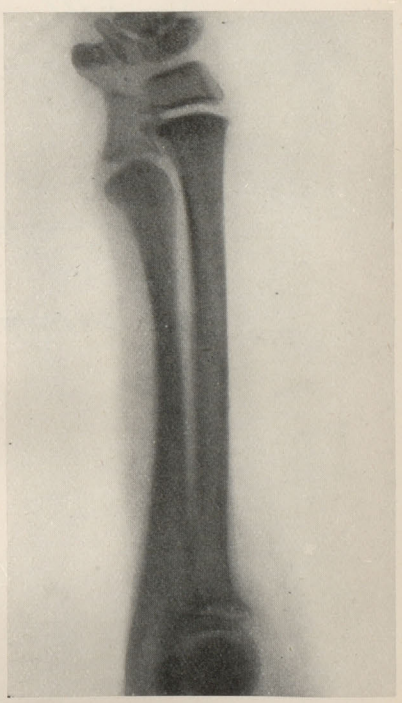


Fig. 12. Exp. 463. Barley.

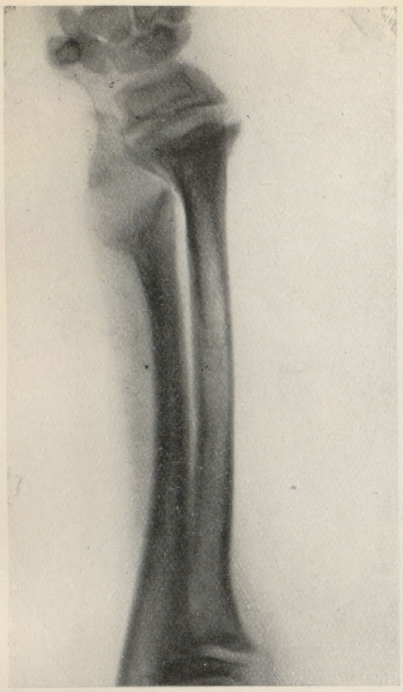


FIG. 13. Exp. 464. Rice (polished) +20 per cent. 'commercial wheat germ'.

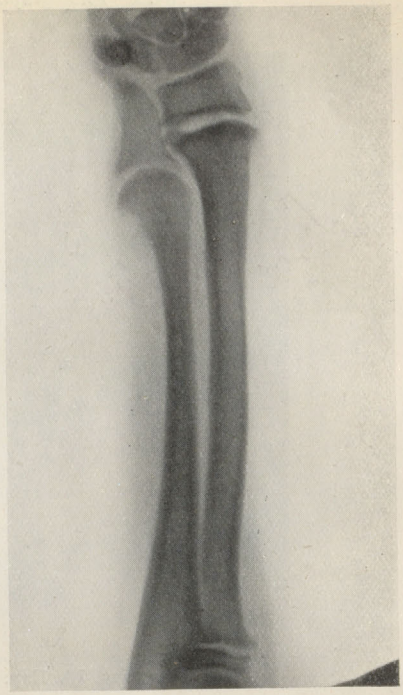


FIG. 14. Exp. 465. Rice (polished).

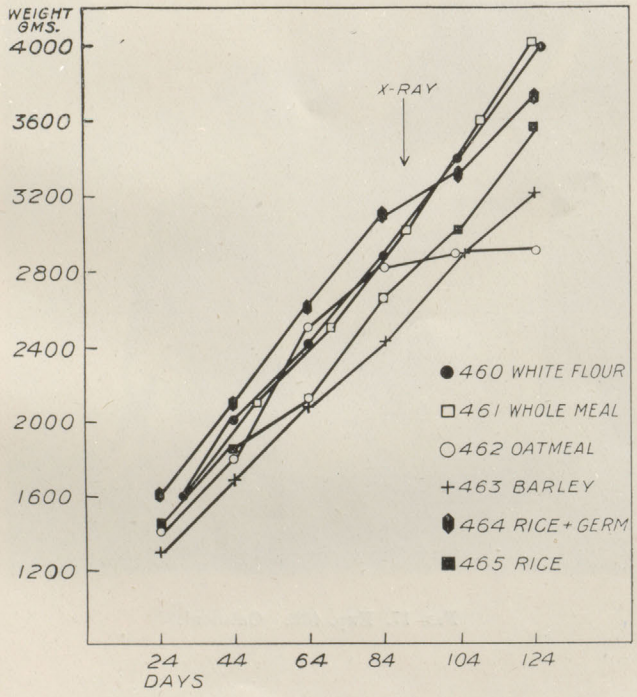


FIG. 15. Weight curves of 460, 461, 462, 463, 464, and 465. The falling out of line of 462 (oatmeal) after 12 weeks of diet is due to the development of severe rickets and consequent loss of appetite.

FIGS. 16–19. Photographs taken after 15 weeks of diet.

FIG. 16. Exp. 460. White flour.

FIG. 17. Exp. 462. Oatmeal.

FIG. 18. Exp. 464. Rice (polished) + 20 per cent. 'commercial wheat germ'.

FIG. 19. Exp. 465. Rice (polished).

FIGS. 20, 21, 22, and 23. Radiographs taken after 17 weeks of diet.

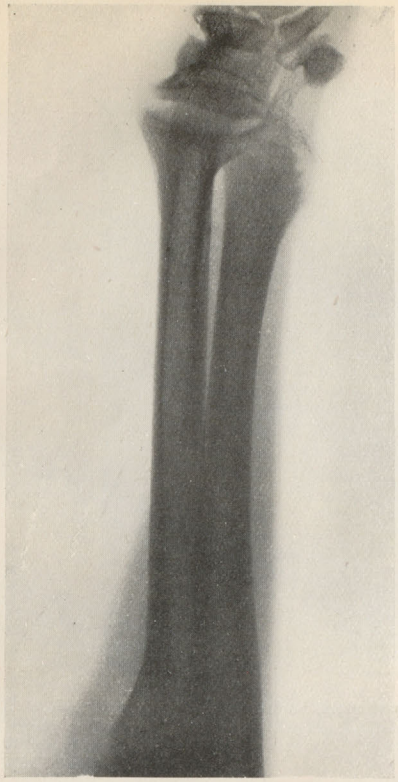


FIG. 20. Exp. 589. Rice.

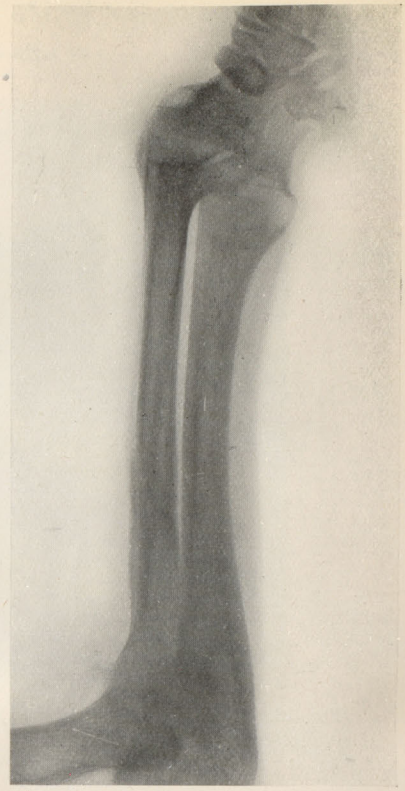


FIG. 21. Exp. 590. Oatmeal.

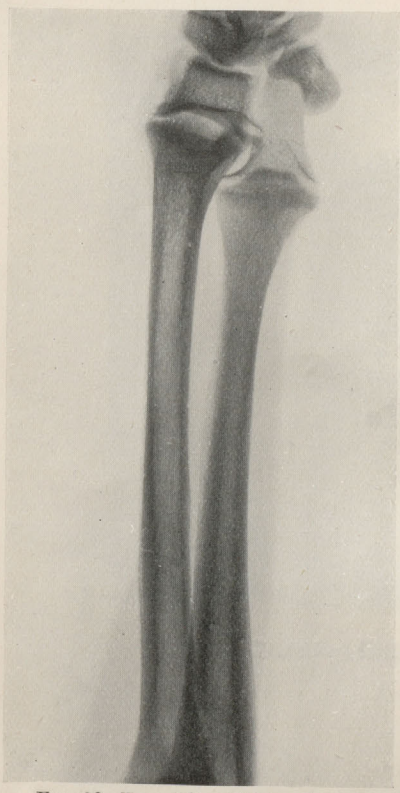


FIG. 22. Exp. 591. White flour.

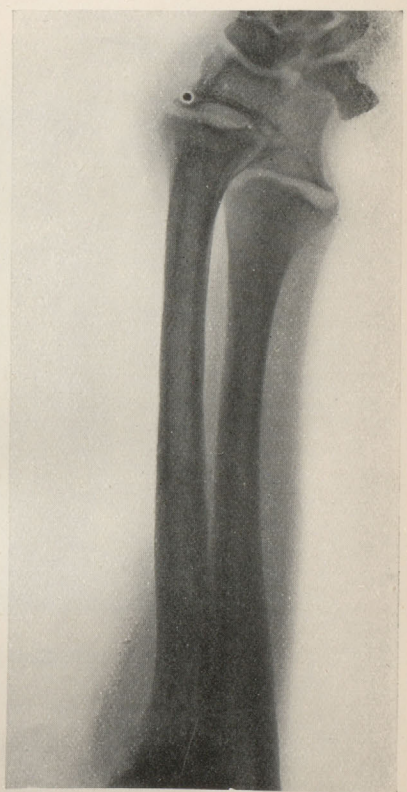


FIG. 23. Exp. 592. White flour and 20 per cent. 'commercial wheat germ'.

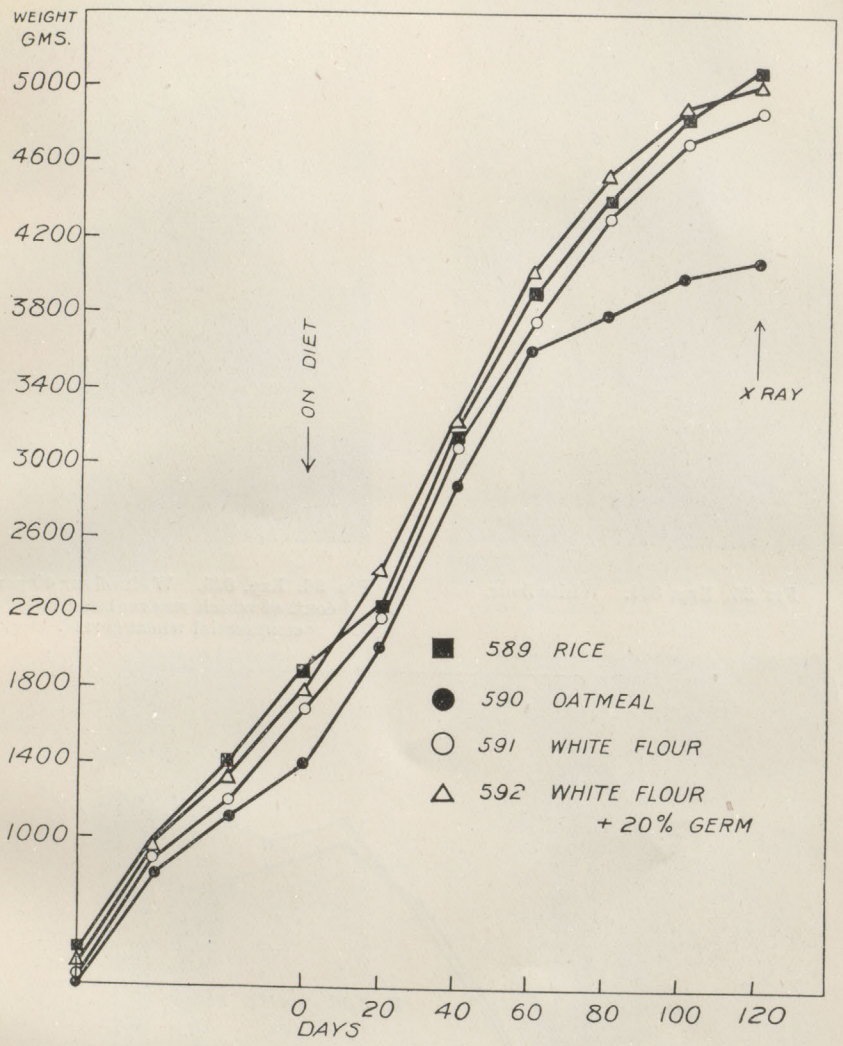


FIG. 24. Weight curves of 589, 590, 591, and 592. Again the oatmeal dog (590) has failed to keep up with the others owing to bad rickets and loss of appetite.

FIGS. 25 and 26. Radiographs taken after 14 weeks of diet.

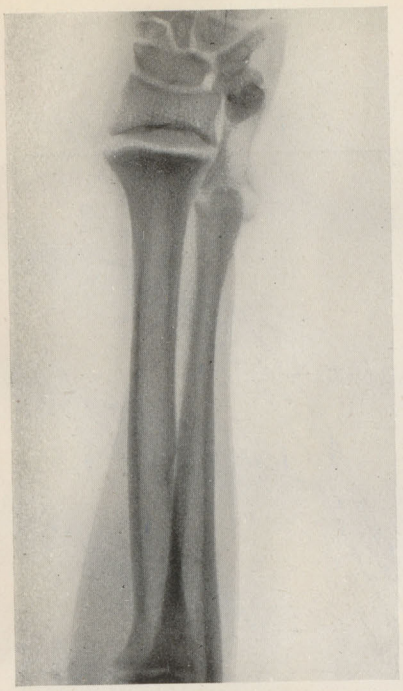


FIG. 25. Exp. 824. White flour.

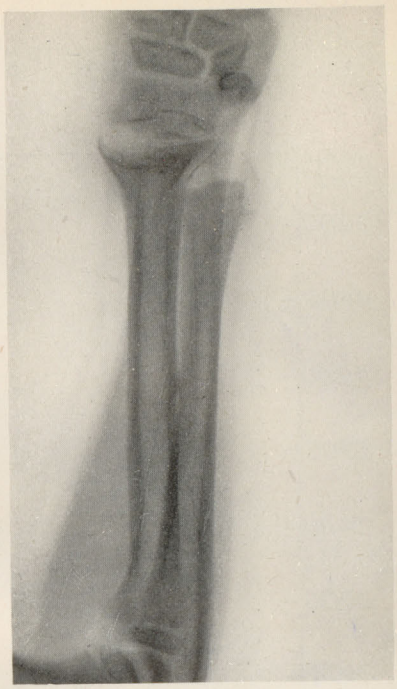


FIG. 26. Exp. 825. White flour 40 per cent. of which was replaced by 'commercial wheat germ'.

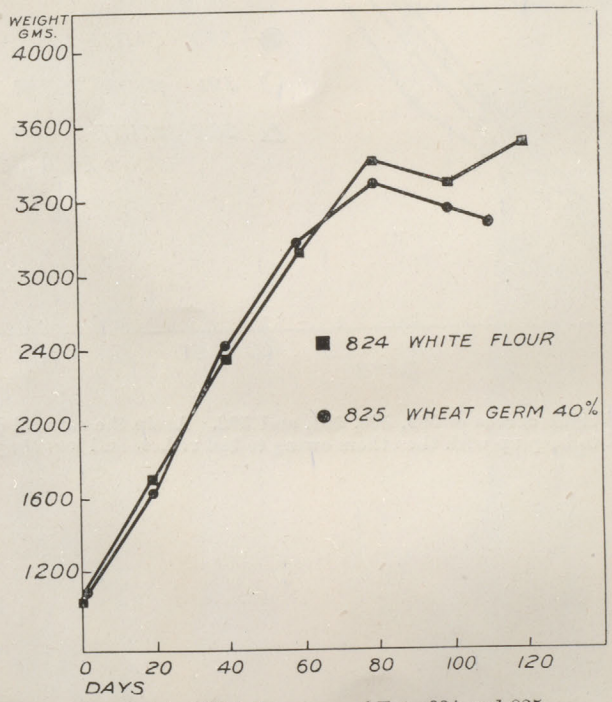


FIG. 26 (a). Weight curves of Exp. 824 and 825.

FIGS. 27–32. Radiographs taken after 9 weeks of diet. 742 FIG. 27 only received 3 c.c. cod-liver oil and 7 c.c. olive oil daily. Others received 10 c.c. olive oil.

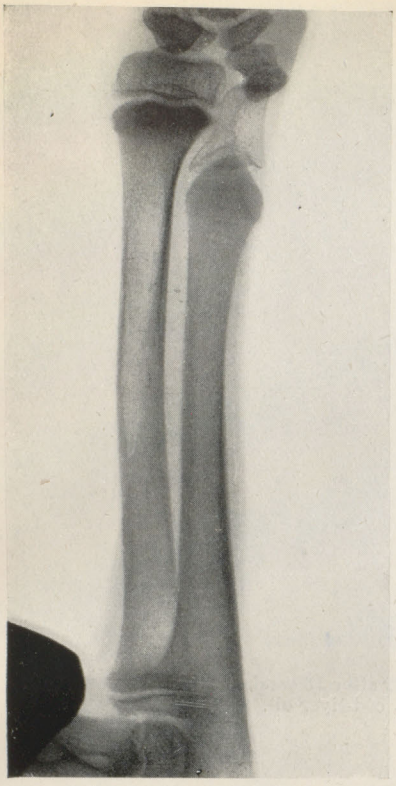


FIG. 27. Exp. 742. Oatmeal and cod-liver oil (3 c.c.)

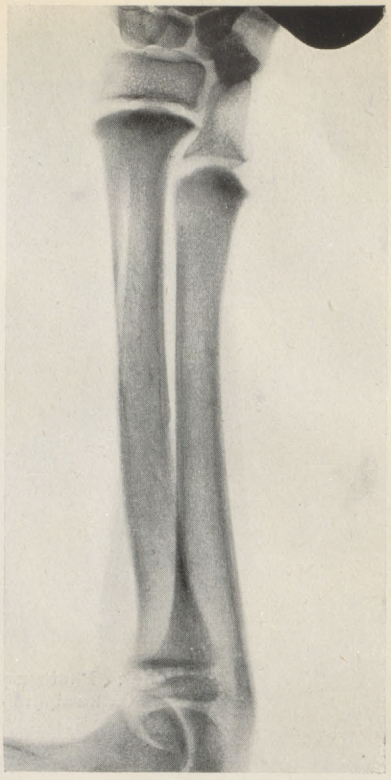


FIG. 28. Exp. 743. White flour.

FIG. 29. Exp. 744. Crushed groats.

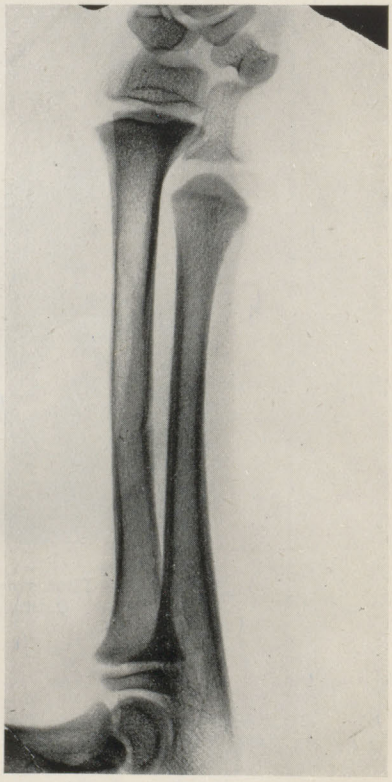


FIG. 30. Exp. 745. Rye.

FIG. 27 (a). Photograph of 742 after 13 weeks of diet. Oatmeal and 3 c.cms. cod-liver oil.

FIG. 31 (a). Photograph of 746 after 13 weeks of diet. Oatmeal and olive oil.

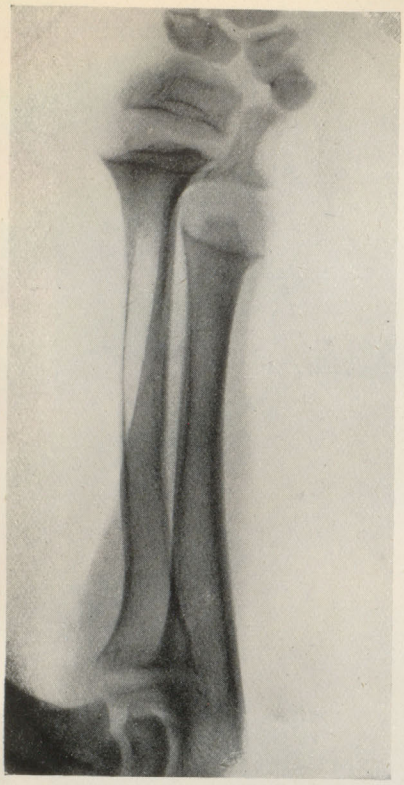


FIG. 31. Exp. 746. Oatmeal.

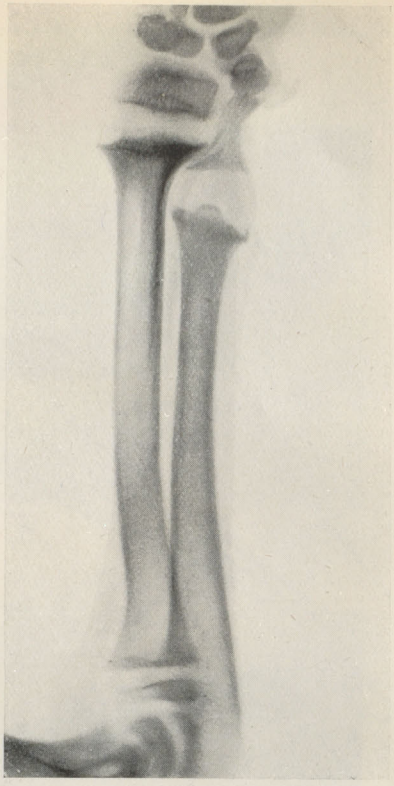


FIG. 32. Exp. 747. Crushed Whole Oats.

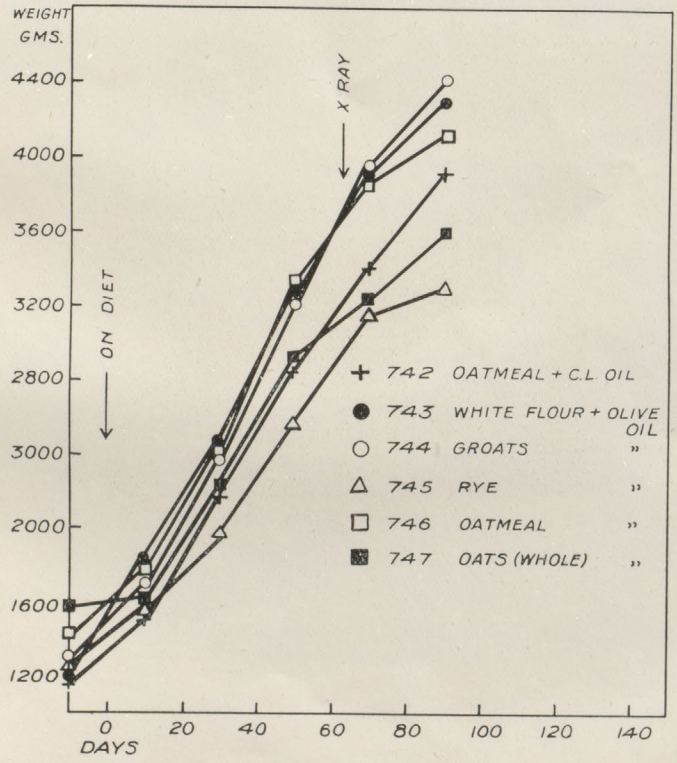


FIG. 33. Weight curves of 742, 743, 744, 745, 746, and 747.

FIGS. 34, 35, 36, 37, and 38. Radiographs of 530, 531, 532, 533, and 534 taken after 20 weeks of diet, i. e. near end of experimental period.

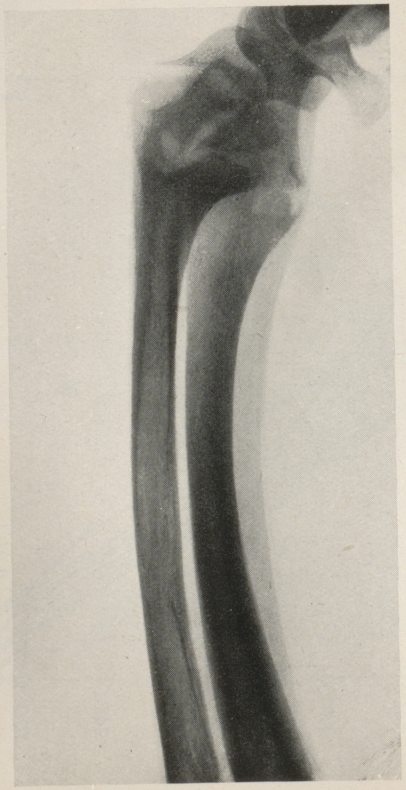


FIG. 34. Exp. 530.
Additional separated milk.
Healing process established.

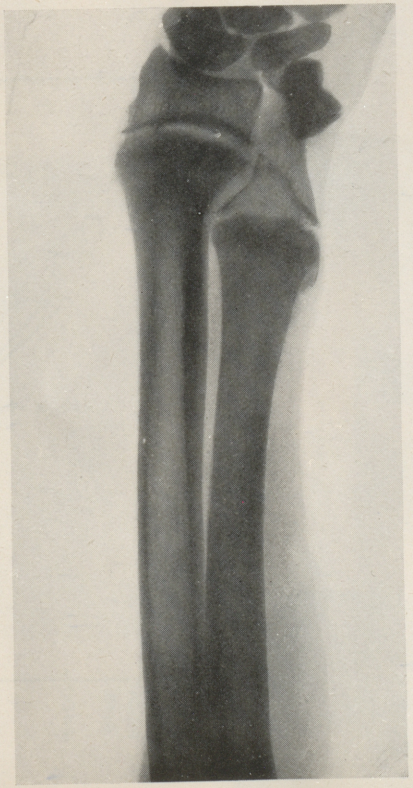


FIG. 35. Exp. 531.
Butter and additional
separated milk.

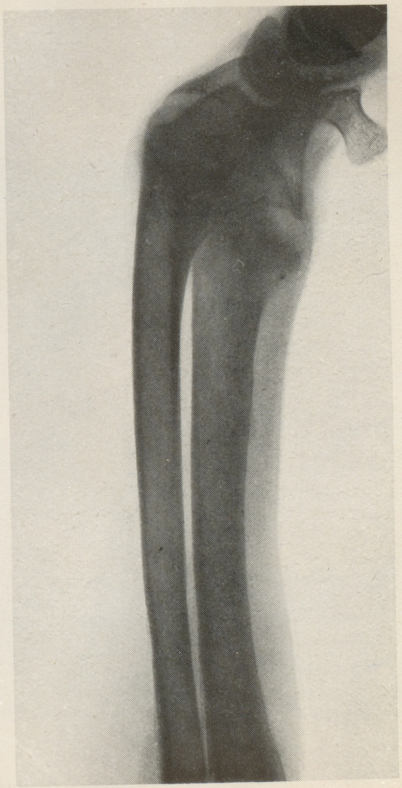


FIG. 36. Exp. 532.
Butter.
Healing established.

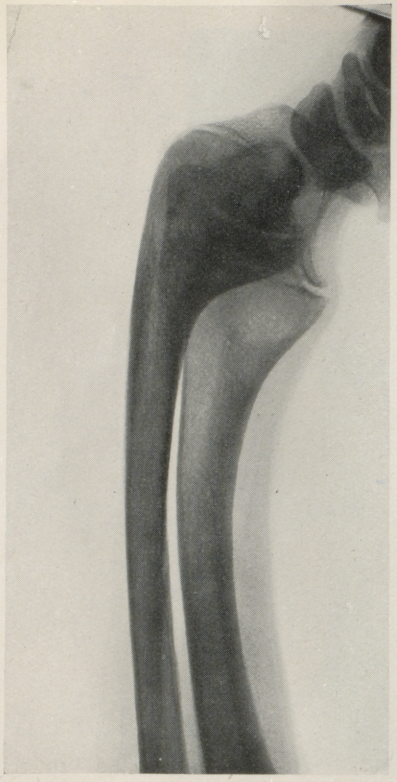


Fig. 37. Olive oil and additional separated milk. Healing changes.

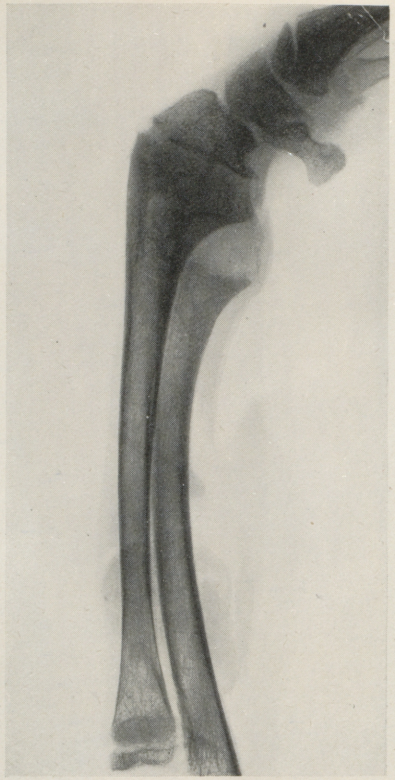


Fig. 38. Control on basal diet only (no fat). Healing.

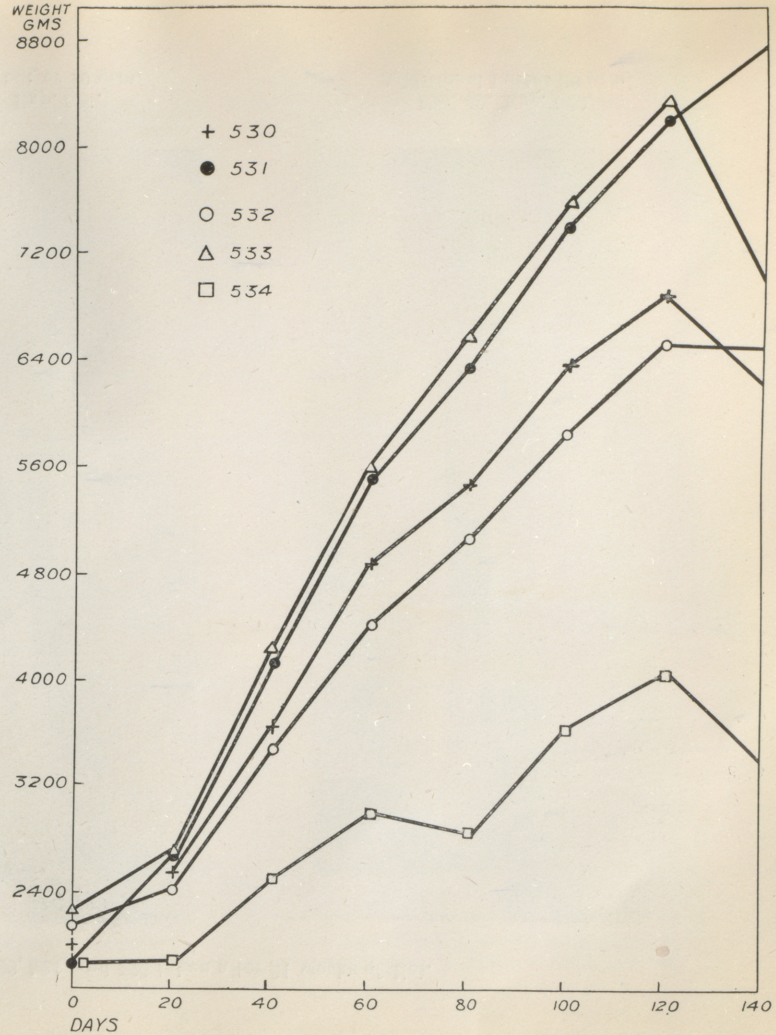


Fig. 39. Weight curves of 530, 531, 532, 533, and 534. The rate of increase in weight of these animals varied because of the differences in amount of protein and energy-bearing substances in the respective diets.

FIGS. 40–45. Radiographs of 520, 521, 522, 523, 524, and 525 taken after 21 weeks of diet.

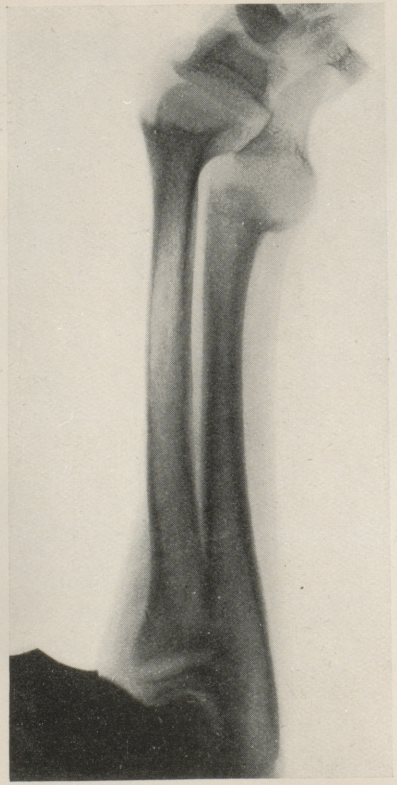


FIG. 40. Exp. 520.
Control on basal diet only (no fat)

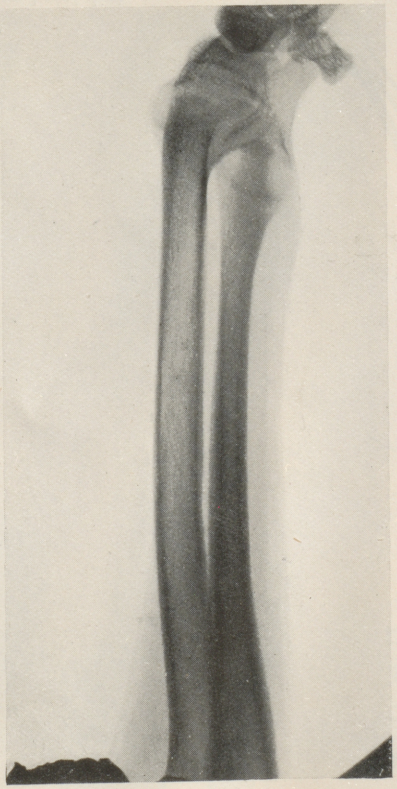


FIG. 41. Exp. 521.
Additional butter 10 grm.

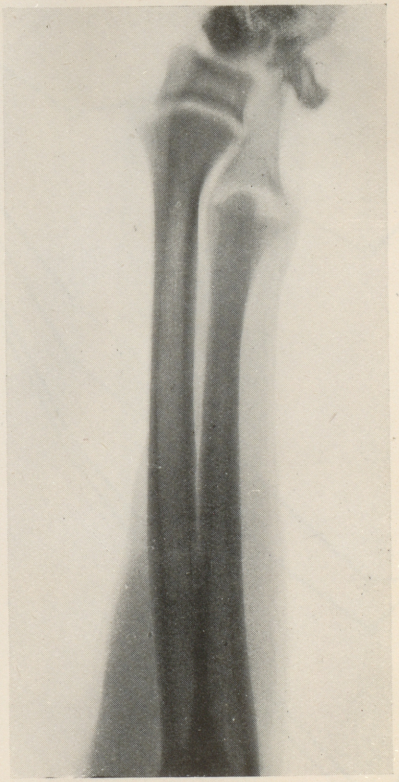


FIG. 42. Exp. 522.
Additional butter 20 grm.

Fig. 40. Exp. 520.
Control on basal diet only (no fat)

Fig. 41. Exp. 52[illegible].
Additional butter 10 grm.

Fig. 42. Exp. 522.
Additional butter 20 grm.

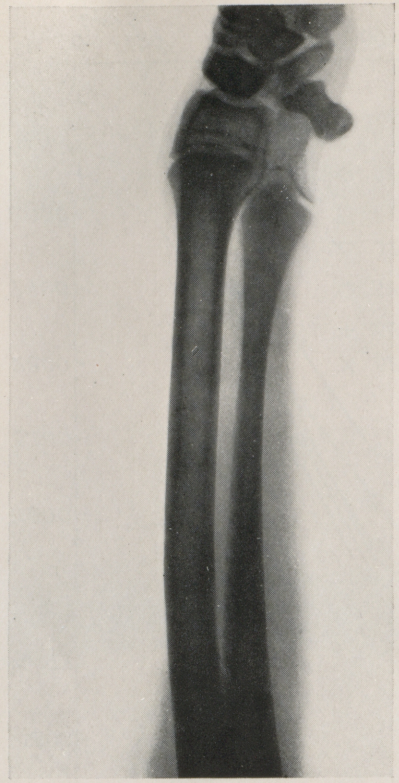


Fig. 43. Exp. 523.
Additional butter 20 grm.
and calcium carbonate 0·85 grm.

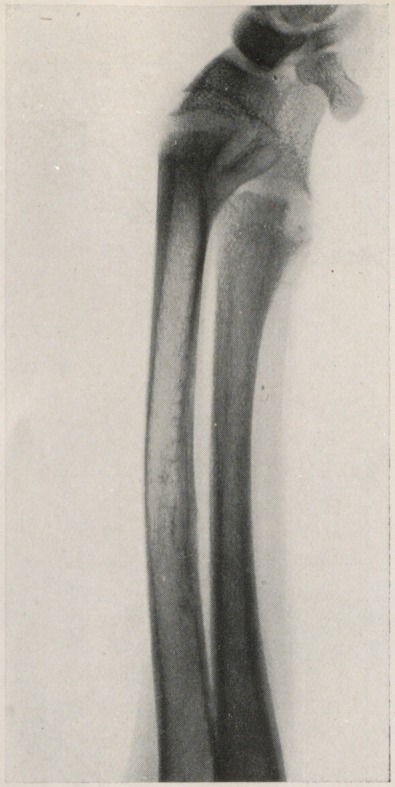


Fig. 44. Exp. 524.
Additional calcium carbonate
0.85 grm. (no fat).

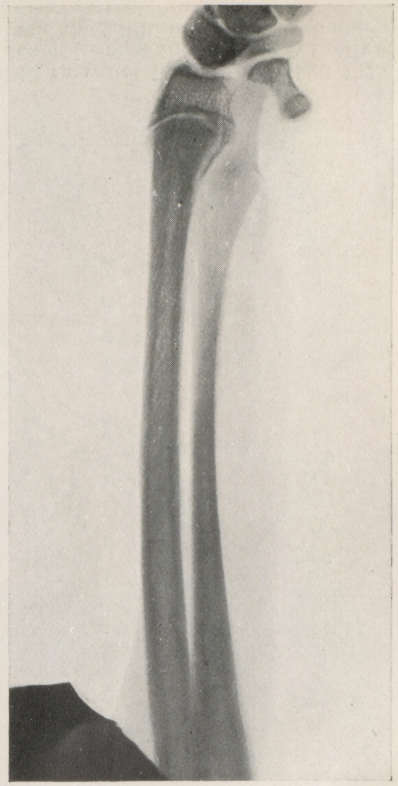


Fig. 45. Exp. 525.
Additional separated milk powder
equivalent to 300 c.c. milk (no fat).

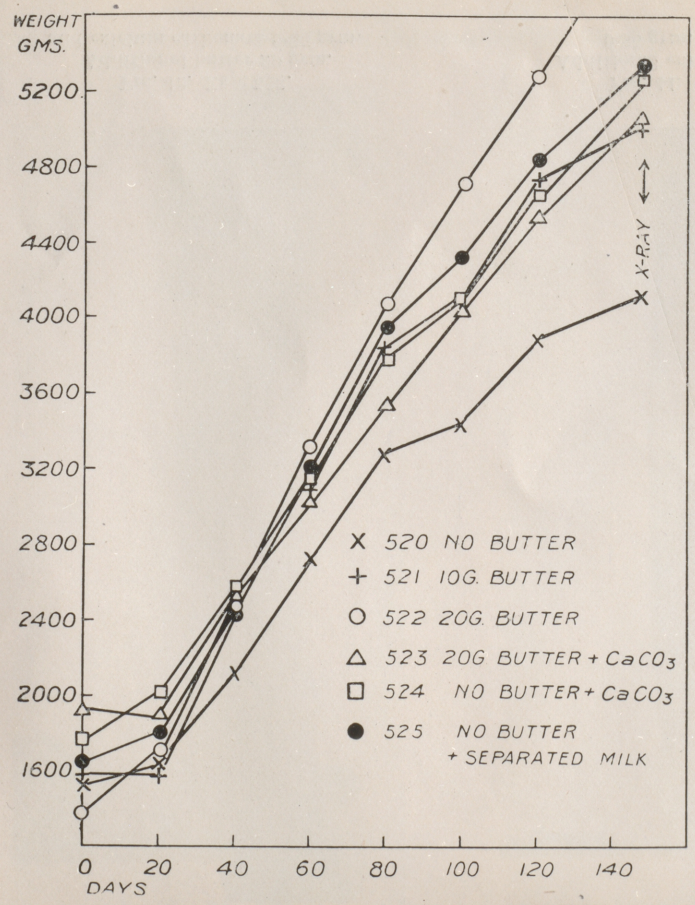


FIG. 46. Weight curves of 520, 521, 522, 523, 524, and 525.

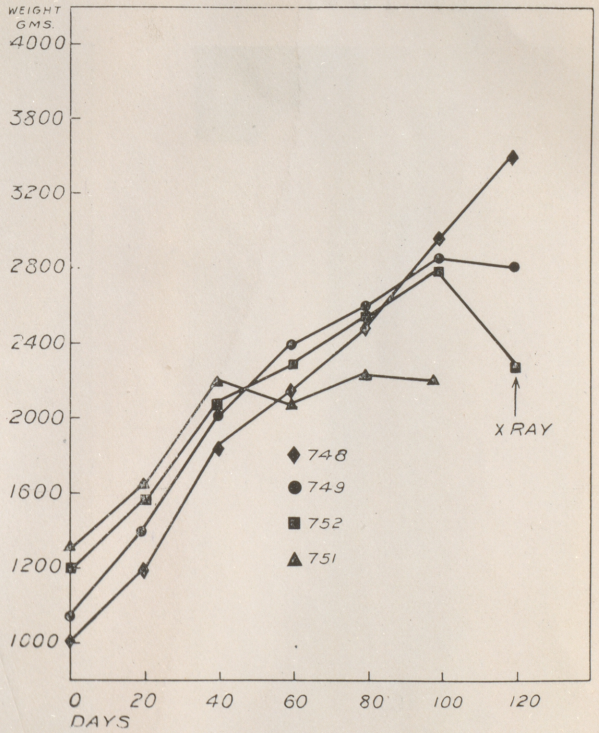


FIG. 51. Weight curves of 748, 749, 751, and 752. Note early cessation of increase in weight of 751 (olive oil) after 6 weeks and the fall in weight of 752 (butter) after 14 weeks of diet.

FIGS. 47–50. Radiographs of 748, 749, 750, and 751 taken after 10 weeks of diet. Cereal in all cases oatmeal.

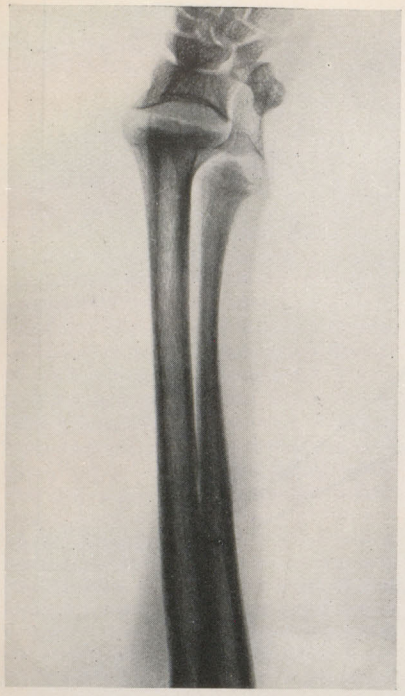


FIG. 47. Exp. 748. Butter and calcium carbonate.

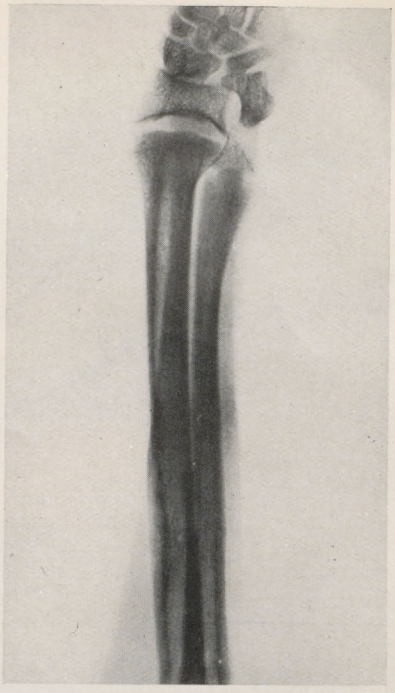


FIG. 48. Exp. 749. Butter and calcium phosphate.

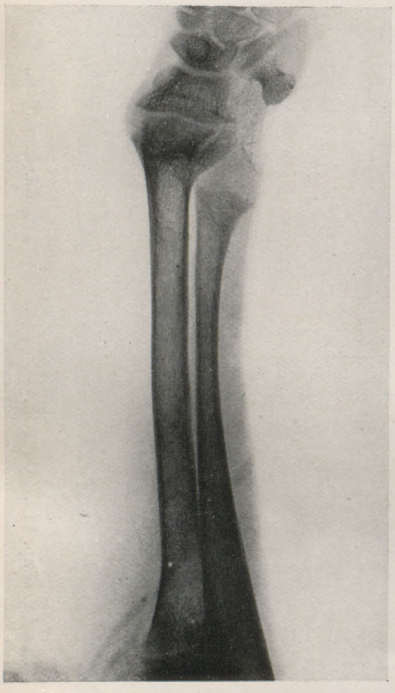


FIG. 49. Exp. 751. Olive oil.

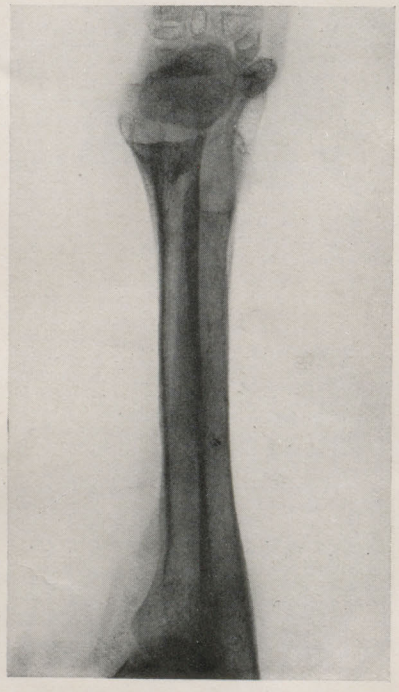


FIG. 50. Exp. 752. Butter.

Fig. 52 and 53. Radiographs of 612 and 613 taken after 17 weeks of diet.

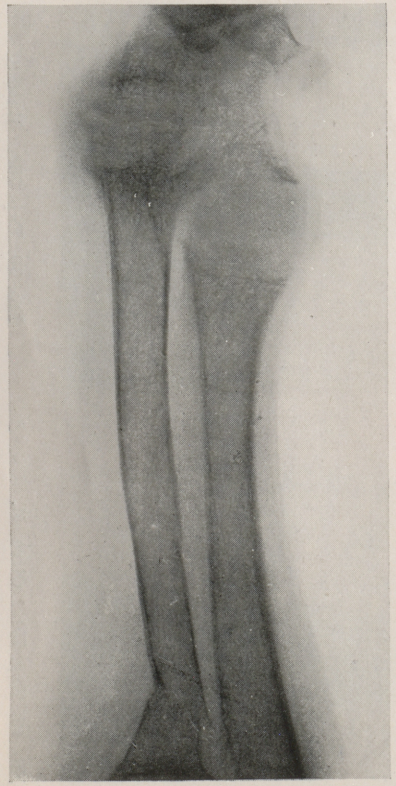


Fig. 52. Exp. 613. Control.

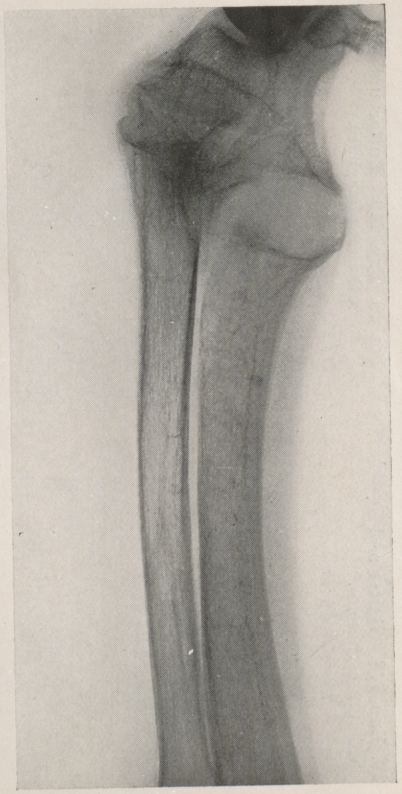


Fig. 53. Exp. 616. Cabbage.

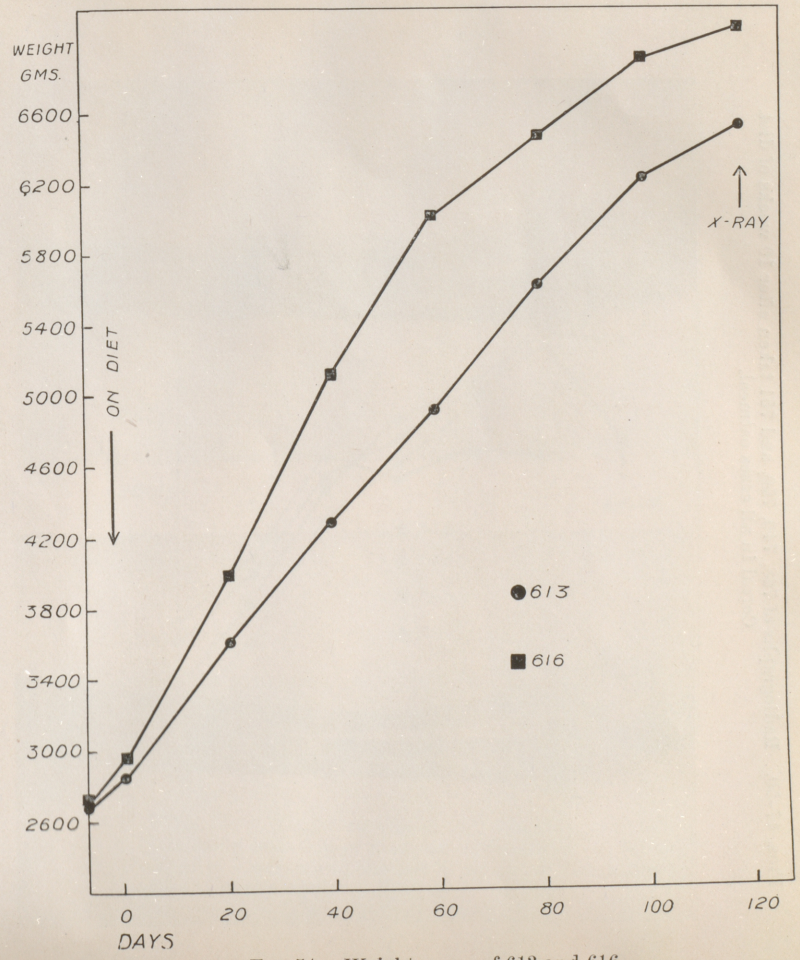


Fig. 54. Weight curve of 613 and 616.

Fig. 52. Exp. 613. Control.

Fig. 53. Exp. 616. Cabbage.

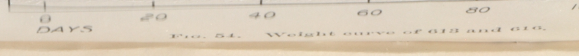


Fig. 54. Weight curve of 613 and 616.

Figs. 55–57. Radiographs of 635, 637, and 638 taken after 33 weeks of diet.

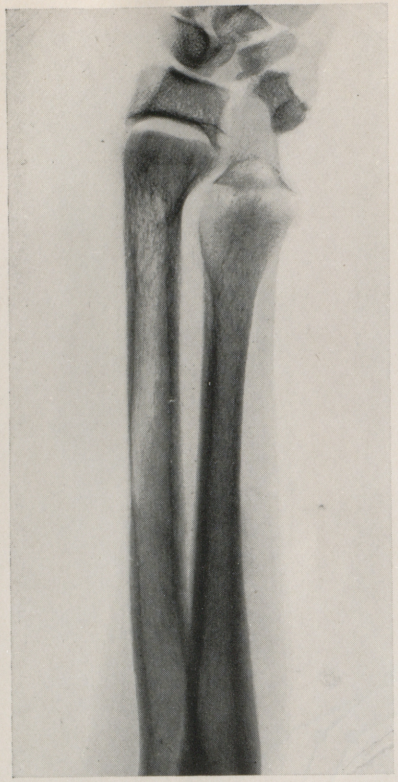


Fig. 55. Exp. 635. Cabbage.
20 to 120 grm. daily.

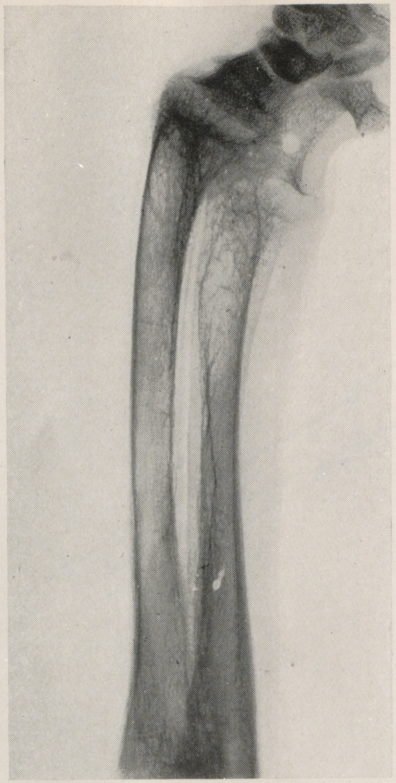


Fig. 56. Exp. 637. Carrot.
20 to 120 grm. daily.

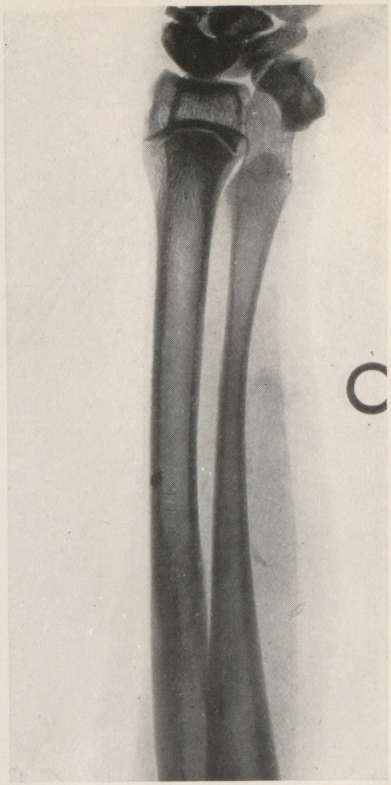


Fig. 57. Exp. 638. Egg-yolk.
$\frac{1}{3}$ to 1 daily.

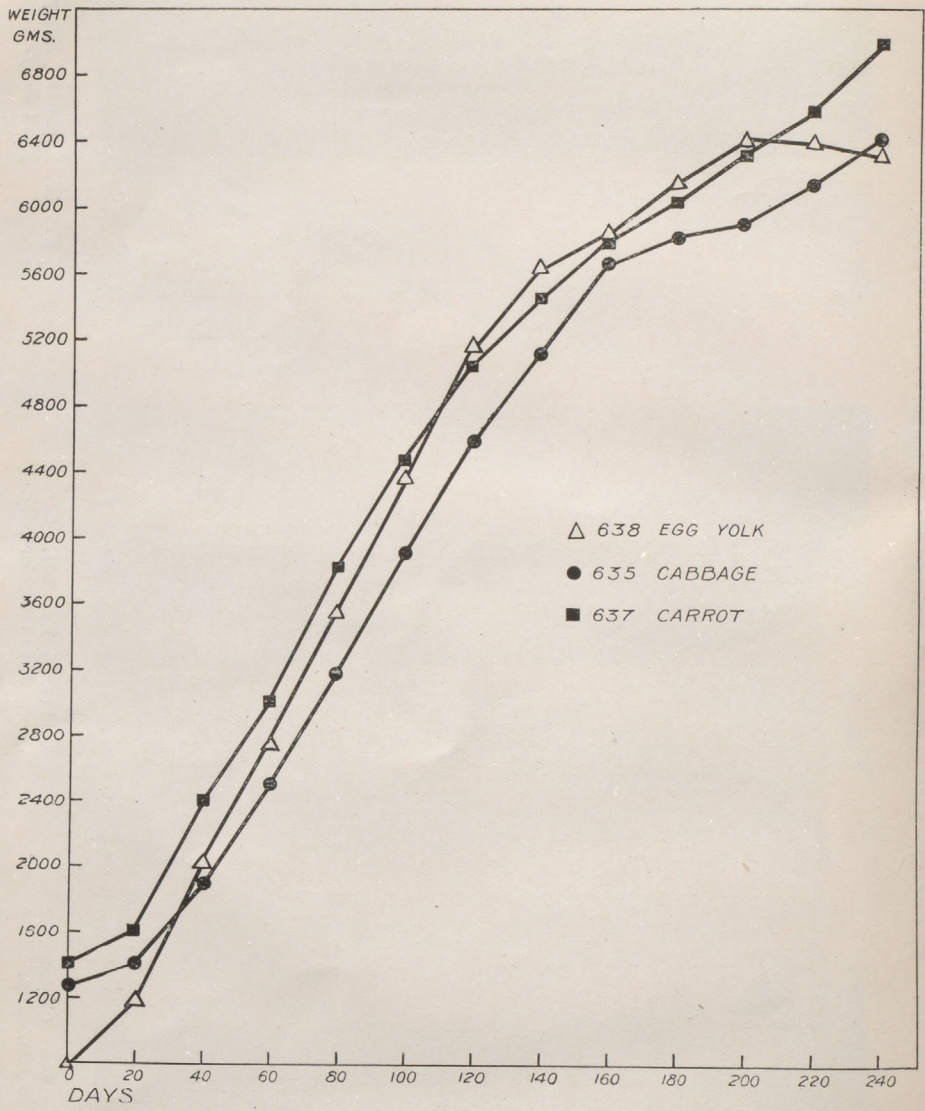


FIG. 58. Weight curves of 635, 637, and 638.

Fig. 59 and 60. Radiographs of 604 and 606 taken after 16 weeks of diet.

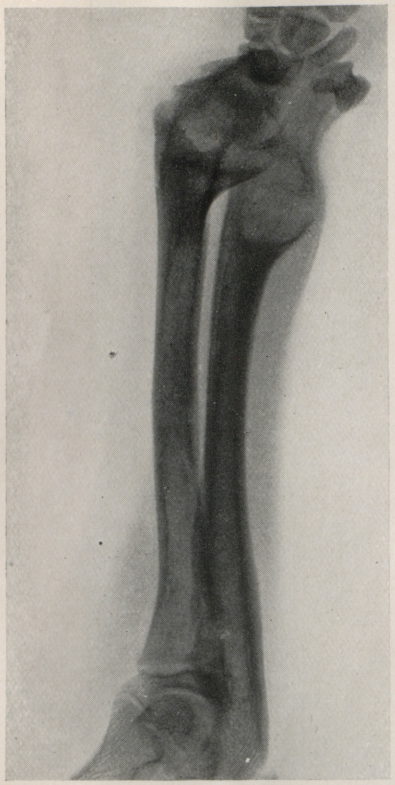


Fig. 59. Exp. 604. Control.

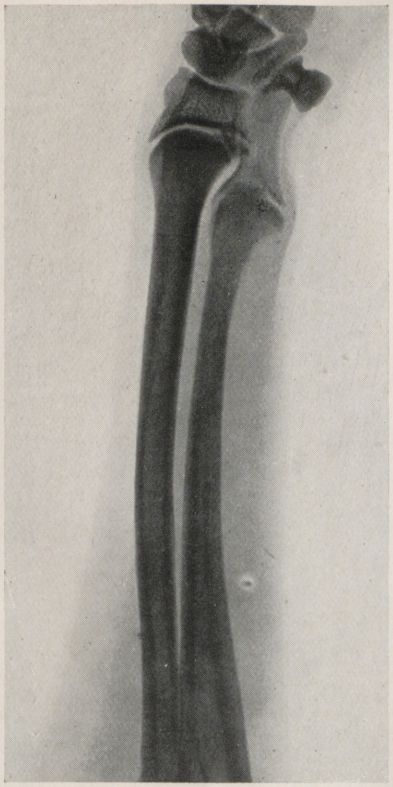


Fig. 60. Exp. 606. Egg-yolk ($\frac{2}{3}$ to 1 daily).

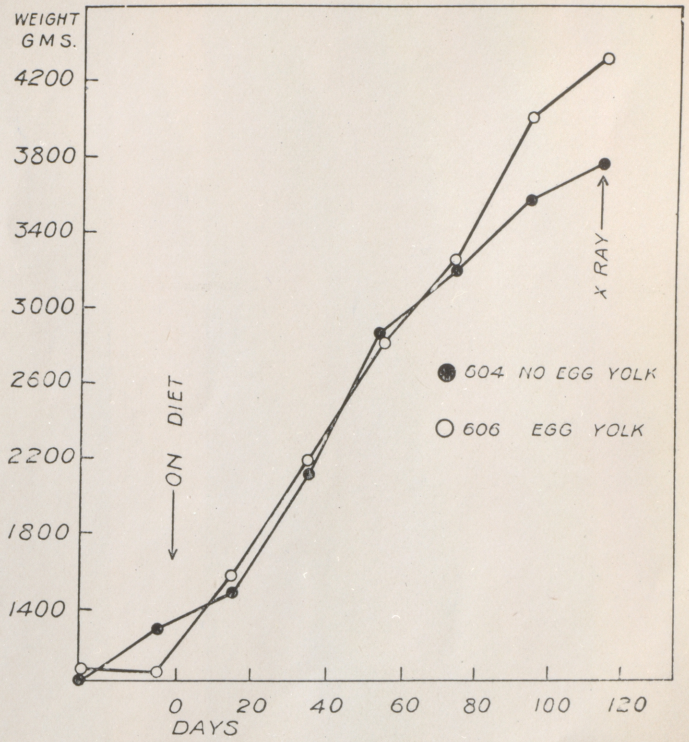


Fig. 61. Weight curves of 604 and 606.

FIGS. 62 to 69. Radiographs of 710, 711, 712, 713, 714, 715, 716, and 717 after 12 weeks of diet. All received oatmeal as cereal and olive oil except 716 (FIG. 68) which received cod-liver oil.

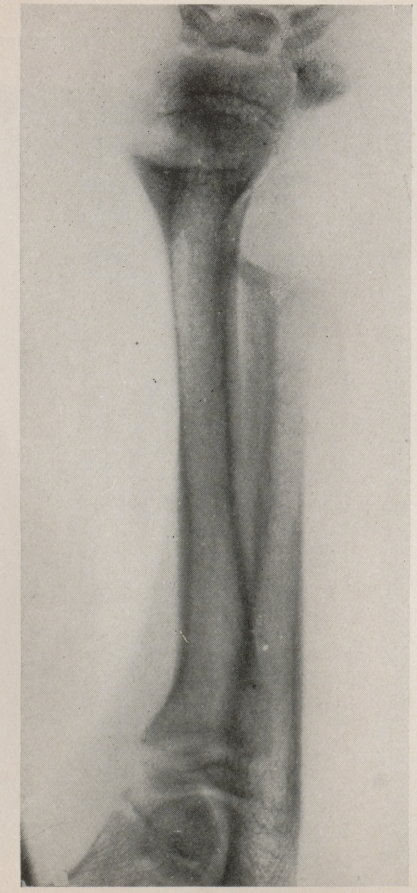


FIG. 62. Exp. 710. Sodium acid phosphate + 2 x grm. oatmeal.

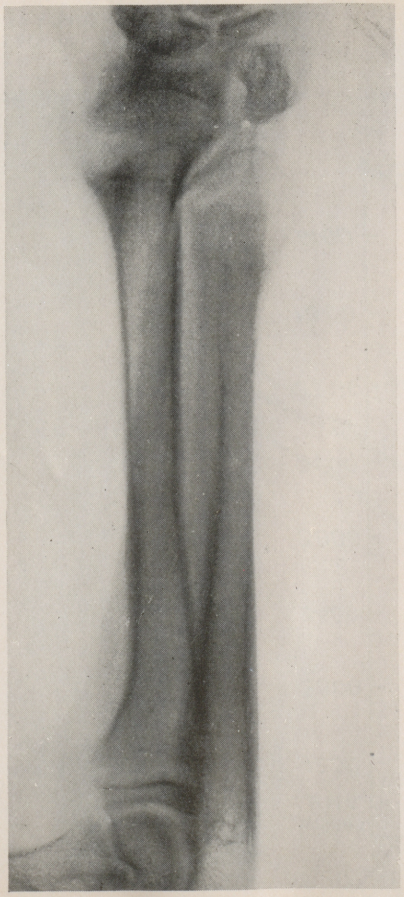


FIG. 63. Exp. 711. Calcium phosphate + 2 x grm. oatmeal.

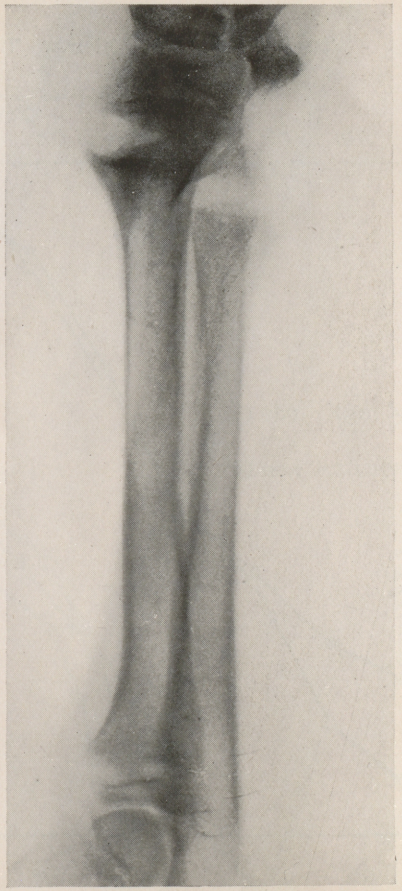


FIG. 64. Exp. 712. x grm. oatmeal.

Fig. 62. Exp. 710. Sodium acid phosphate + 2 x grm. oatmeal.

Fig. 63. Exp. 711. Calcium phosphate + 2 x grm. oatmeal.

Fig. 64. Exp. 712. x grm. oatmeal.

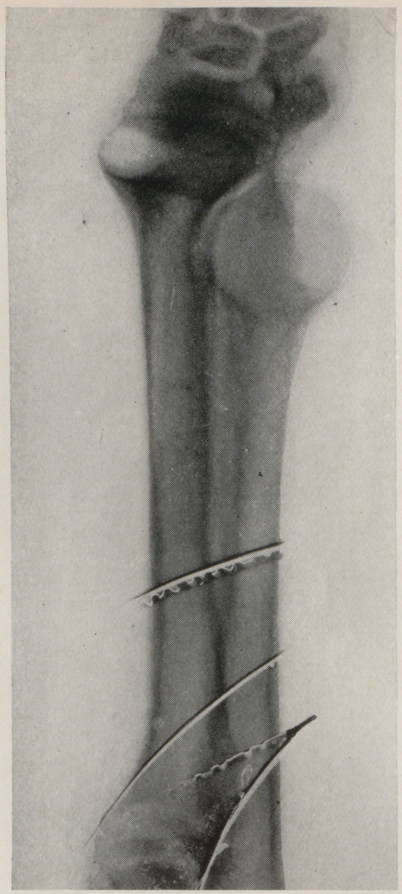


Fig. 65. Exp. 713. 2 x grm. oatmeal.

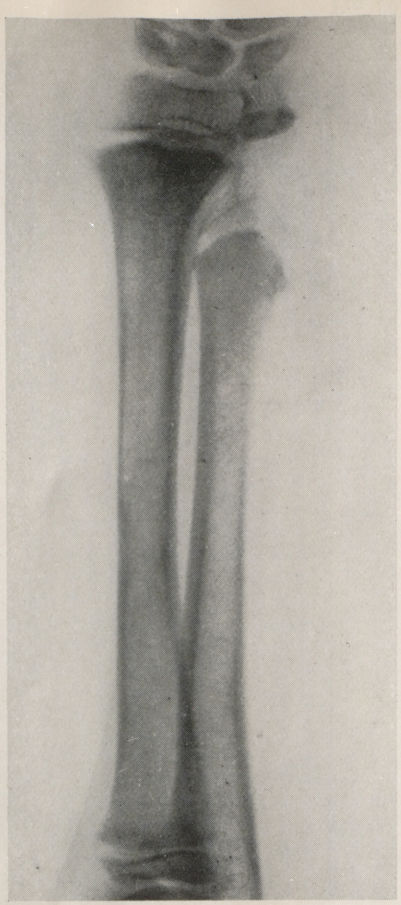


Fig. 66. Exp. 714. Ultra-violet Radiations and x oatmeal.

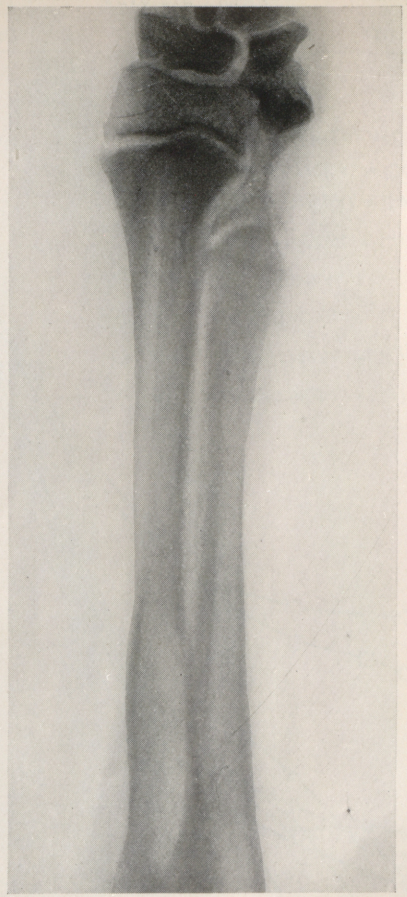


Fig. 67. Exp. 715. Calcium carbonate and 2 x grm. oatmeal. See also Fig. 70 and 72.

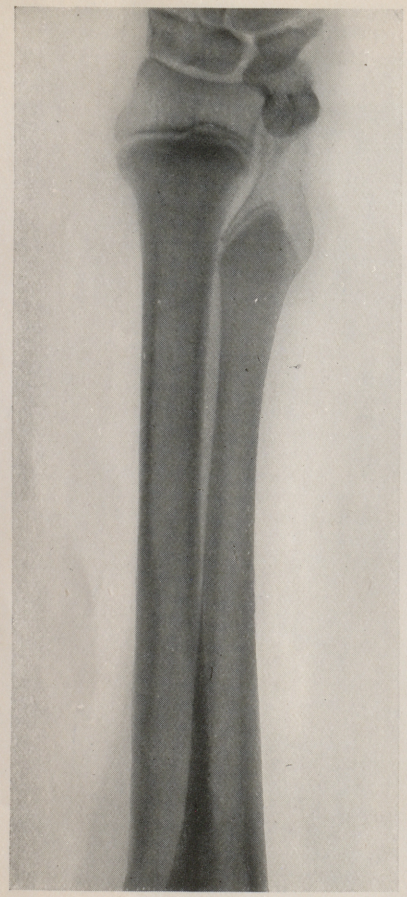


FIG. 68. Exp. 716.
Cod-liver oil and 2 x oatmeal.
See also FIGS. 71 and 73.

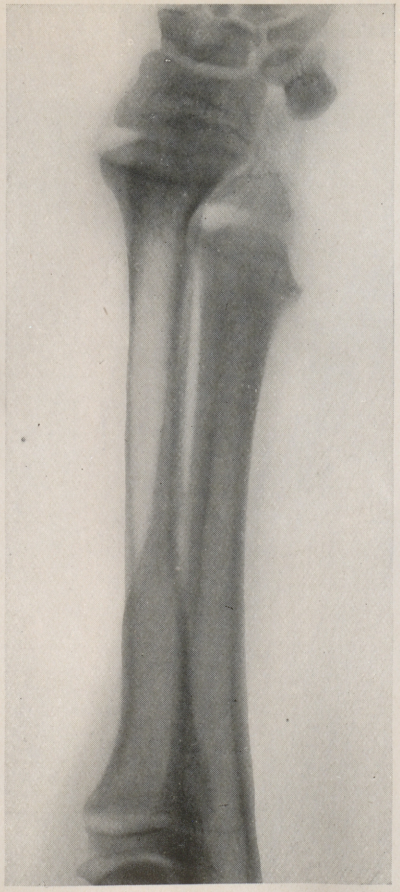


FIG. 69. Exp. 717.
Ultra-violet Radiations and 2 x grm oatmeal.

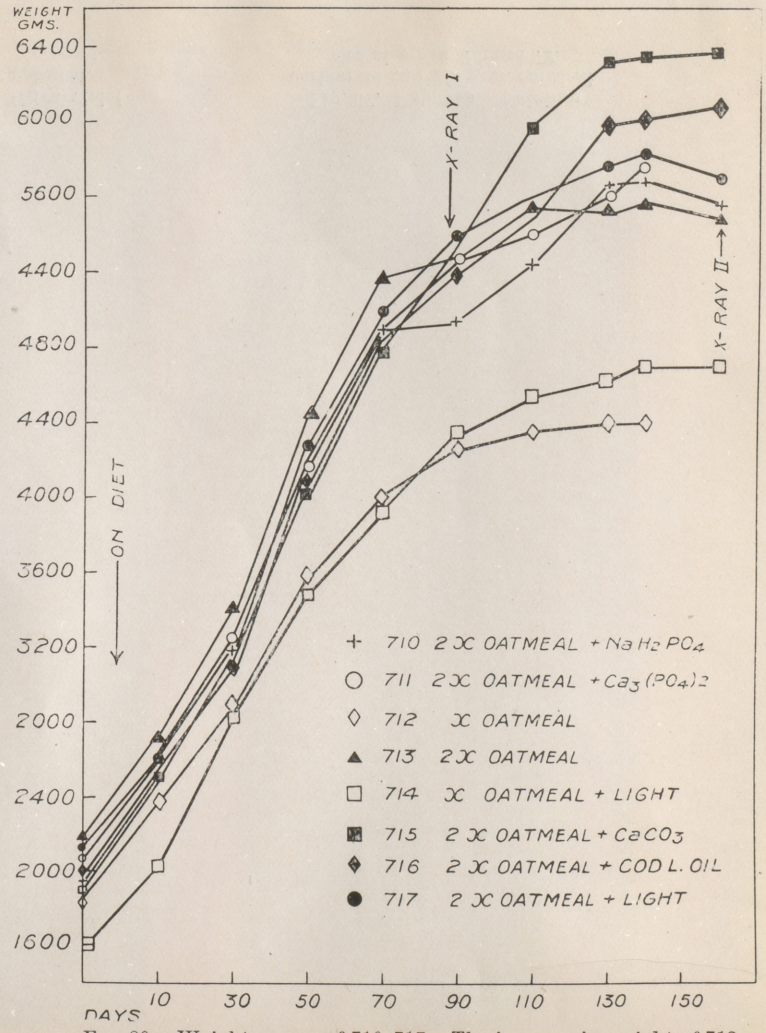


FIG. 80. Weight curves of 710–717. The increase in weight of 712 and 714 is less than that of the other animals of the litter because they received only half the oatmeal.

Cod-liver oil and 2 x oatmeal.
See also Figs. 71 and 73.

Ultra-violet Radiation
oatmeal.

DAYS 10 30 50 70 90 110 130 150

Fig. 80. Weight curves of 710–717. The increase in weight of 712 and 714 is less than that of the other animals of the litter because they received only half the oatmeal.

Radiographs of 715 and 716 taken after 23 weeks of diet.

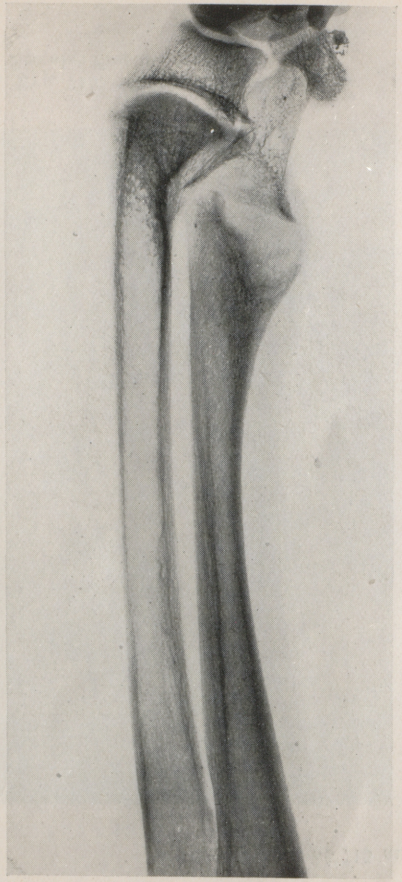


Fig. 70. Exp. 715. Calcium carbonate.
See also Figs. 67 and 72.

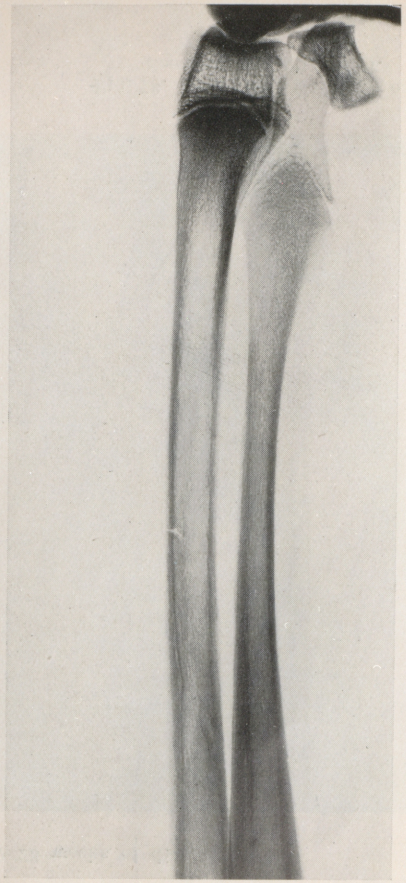


Fig. 71. Exp. 716. Cod-liver oil.
See also Figs. 68 and 73.

Photographs of 715 and 716 taken after 23 weeks of diet.

FIG. 72. Exp. 715. Calcium carbonate.
See also FIGS. 67 and 70.

FIG. 73. Exp. 716. Cod-liver oil.
See also FIGS. 68 and 71.

FIGS 74–79. Photomicrographs of costo-chondral junctions of 712, 713, 714, 715, 716, 717 (Magnification × 6).

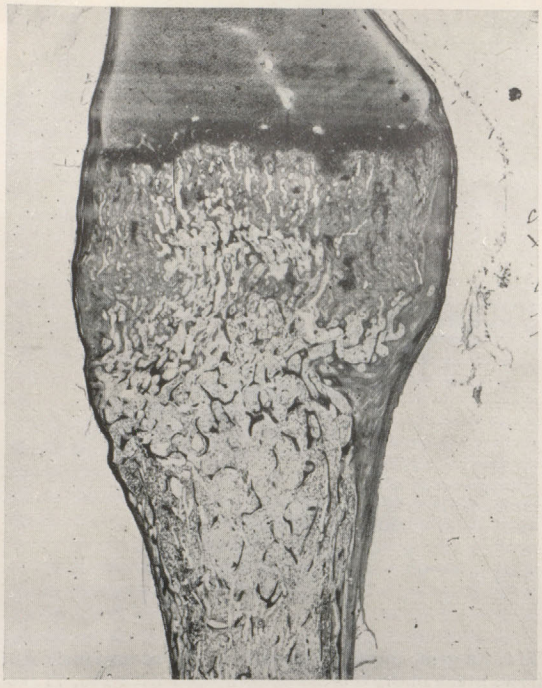


FIG. 74. Exp. 712. x grm. oatmeal (see also FIG. 64).

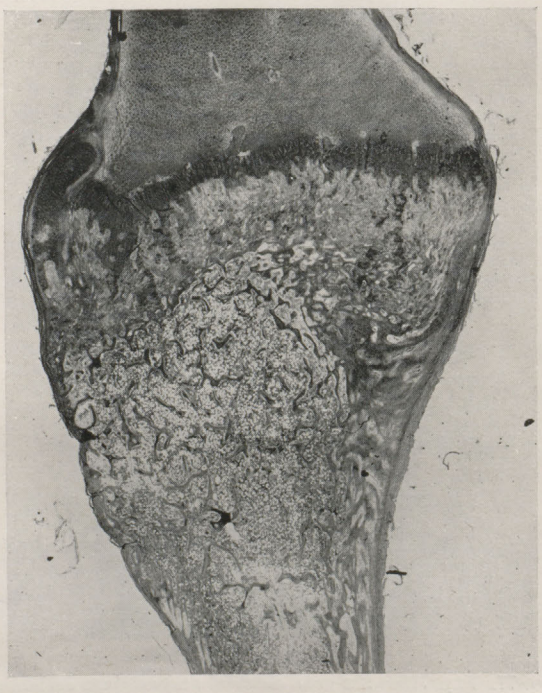


FIG. 75. Exp. 713. 2 x grm. oatmeal (see also FIG. 65).

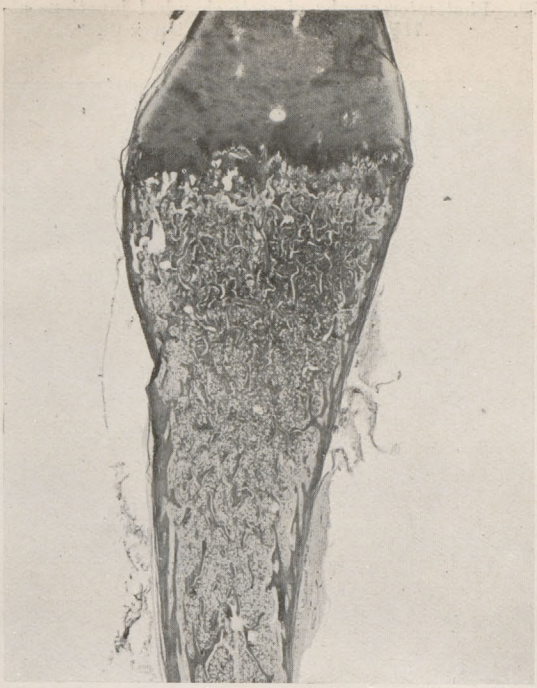


FIG. 76. Exp. 714. x grm. oatmeal and ultra-violet radiations. Although rachitic bone shows great improvement as compared with FIG. 74 (control).
(See also FIG. 66.)

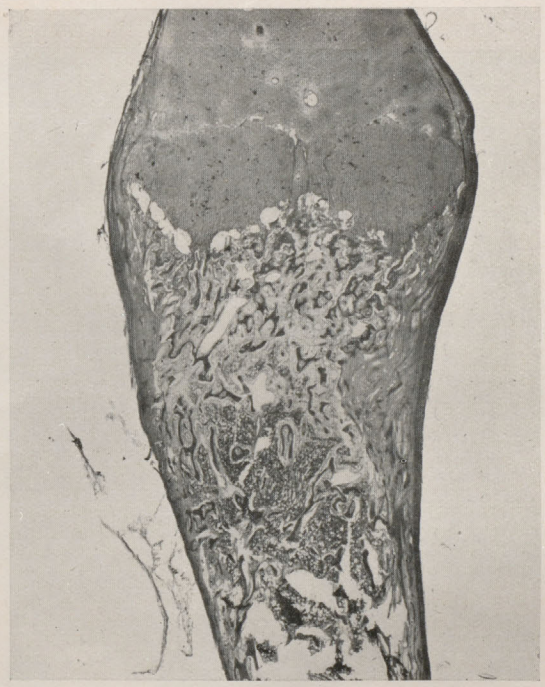


FIG. 77. Exp. 715. 2 x grm. oatmeal and calcium carbonate.
(See also FIGS. 67, 70, and 72.)

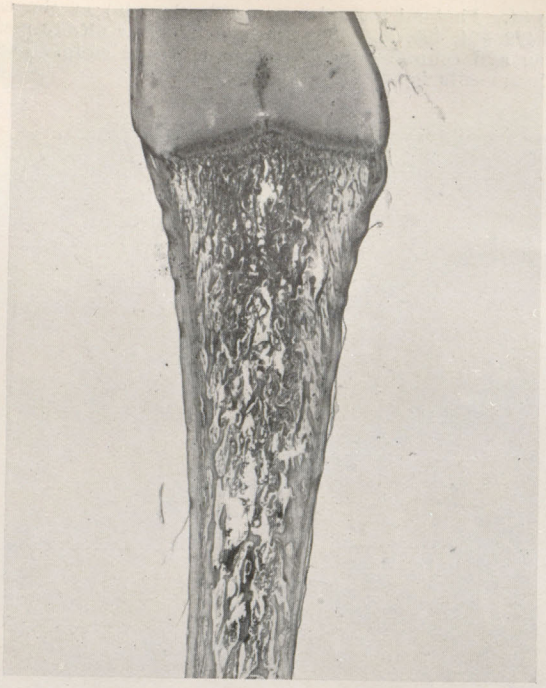


FIG. 78. Exp. 716. 2 x grm. oatmeal and cod-liver oil (see also FIGS. 68, 71, and 73) The most normal junction of the series.

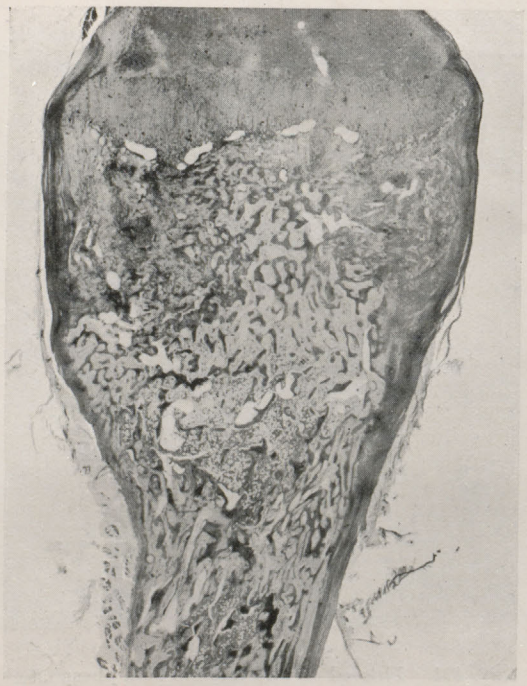


FIG. 79. Exp. 717. 2 x grm. oatmeal and ultra-violet radiations (see also FIG. 69). Is not much better than 713 (Control) FIG. 75 in spite of radiations.

FIGS. 81 to 84. Photomicrographs (magnification 40) of the costo-chondral junctions of 492, 494, 495, and 496. Stained by silver nitrate method without decalcification and counterstained haematoxylin and cosin. The black stain indicates calcium salts deposited in the growing bone.

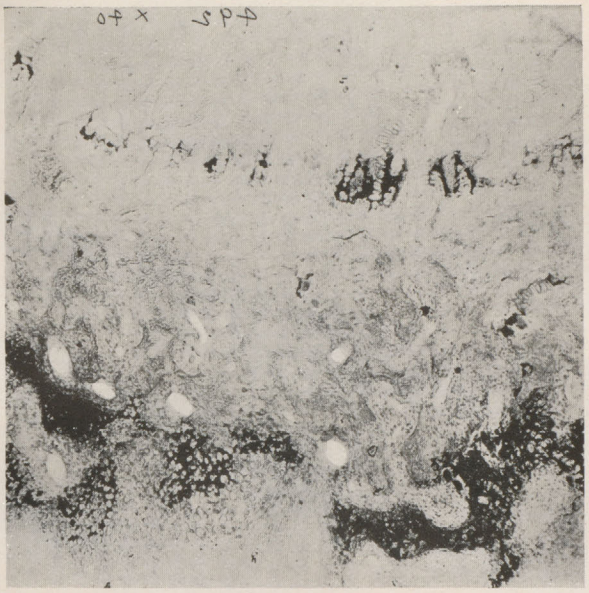


FIG. 81. Exp. 492. Linseed oil and minimum calcium. Rickets and osteoporosis, but attempt at healing visible owing to cessation of growth in last five weeks of experiment.

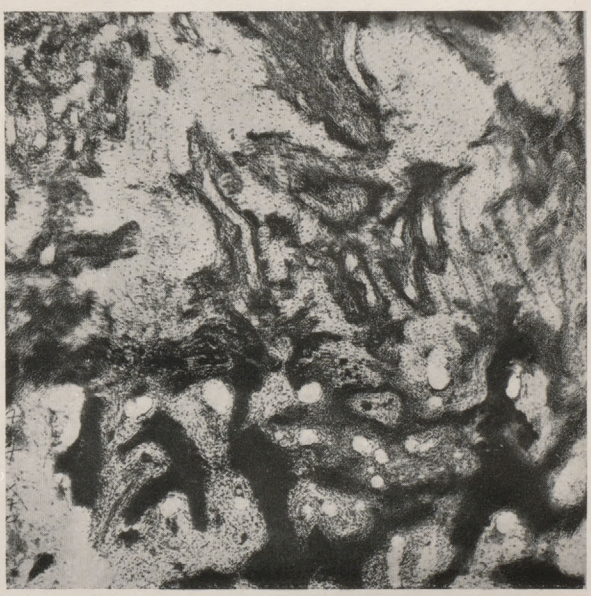


FIG. 82. Exp. 494. Linseed oil and calcium acid phosphate. Rickets.

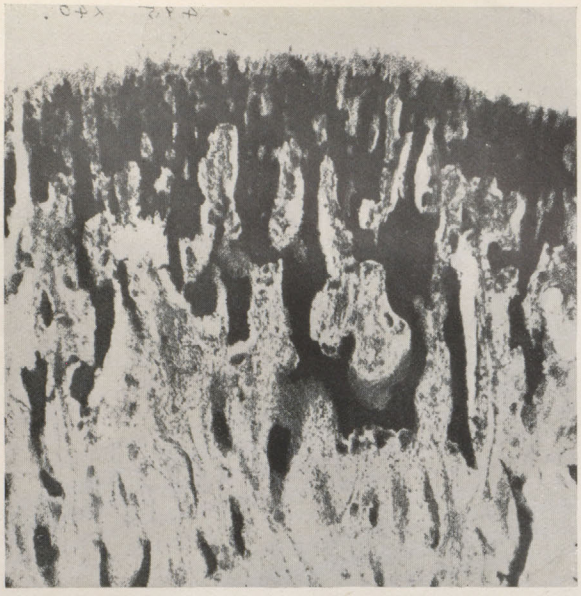


FIG. 83. Exp. 495. Cod-liver oil and minimum calcium. Fairly good calcification at growing edge but very little calcium in deeper trabeculae.

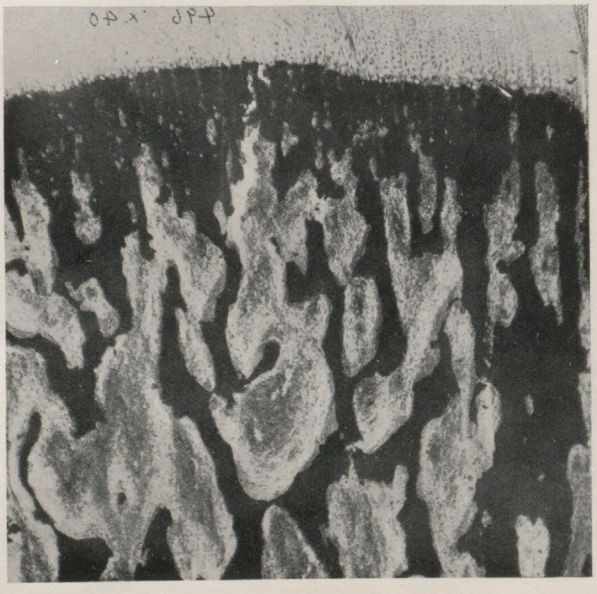


FIG. 84. Exp. 496. Cod-liver oil and calcium acid phosphate. Good calcification.

FIGS. 85 to 90. Effect of saponifiable and unsaponifiable substances of oatmeal and wheat germ. Radiographs of 802, 803, 804, 805, 806, and 807 after 12½ weeks of diet.

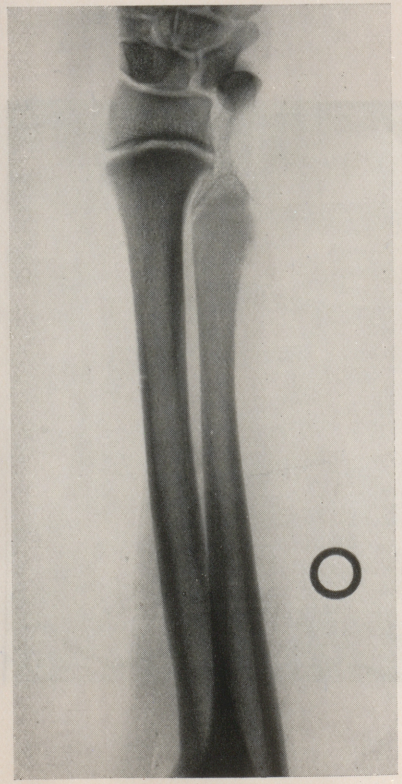


FIG. 85. Exp. 802.
Unsaponifiable substances
from germ.

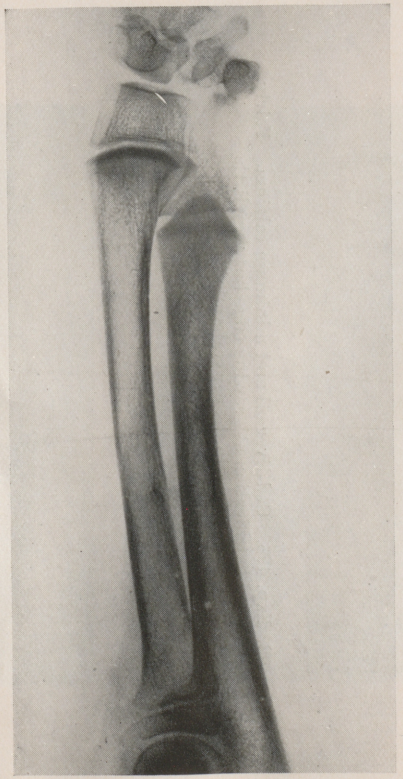


FIG. 86. Exp. 803.
Saponifiable substances
from germ.

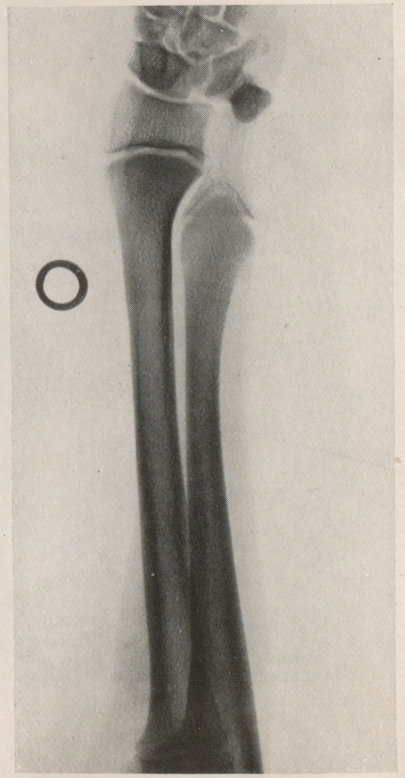


FIG. 87. Exp. 804.
Unsaponifiable substances
from oatmeal.

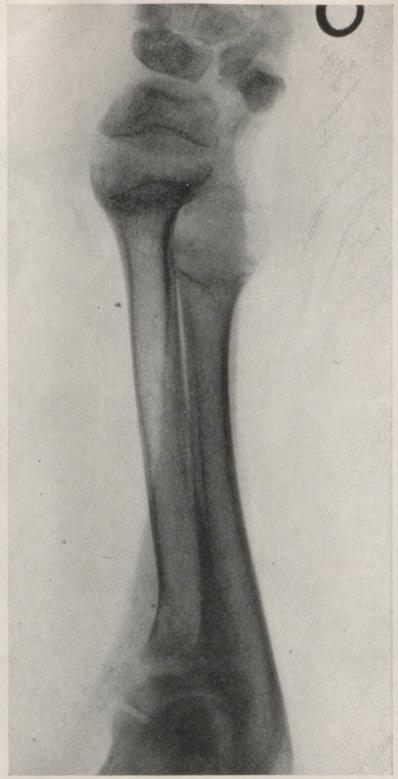


FIG. 88. Exp. 805.
Saponifiable substances from oatmeal.

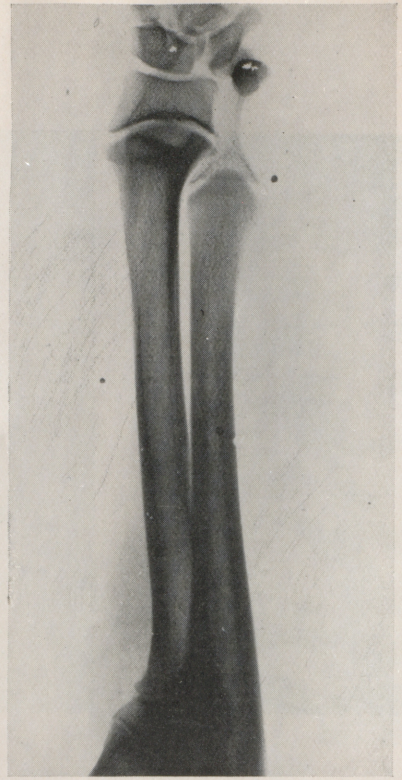


FIG. 89. Exp. 806.
Control (white flour).

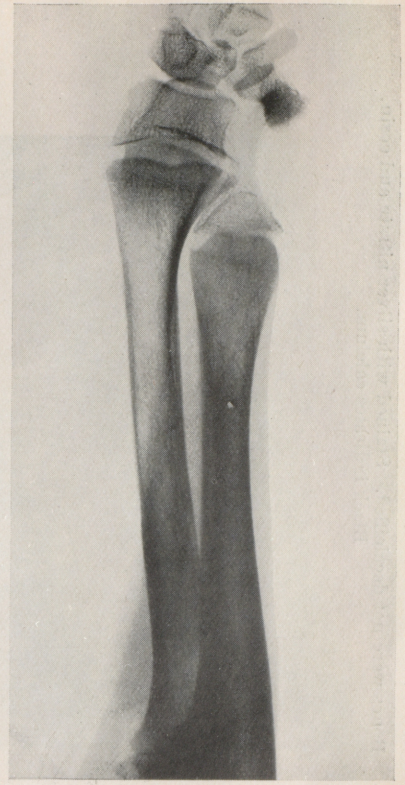


FIG. 90. Exp. 807.
60 per cent. white flour and
40 per cent. 'commercial wheat germ'.

FIGS. 91 to 93. Photomicrographs (magnification 40) of the costo-chondral junctions of 806, 805, and 807.

Bones were not decalcified. Stained with silver nitrate and eosin. Black indicates calcium.

FIG. 91. Exp. 806. Control (white flour). See also FIG. 89. Slightly abnormal calcification and growth.

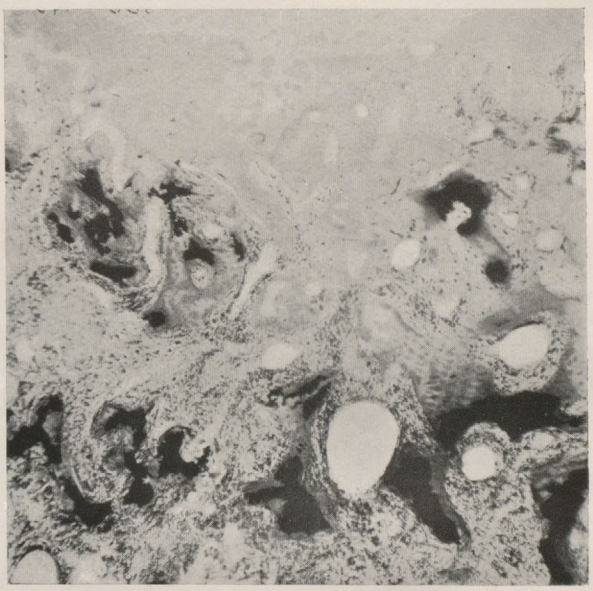


FIG. 92. Exp. 805. Saponifiable substances from oatmeal. See also FIG. 88. Cessation of calcification and great derangement at junction.

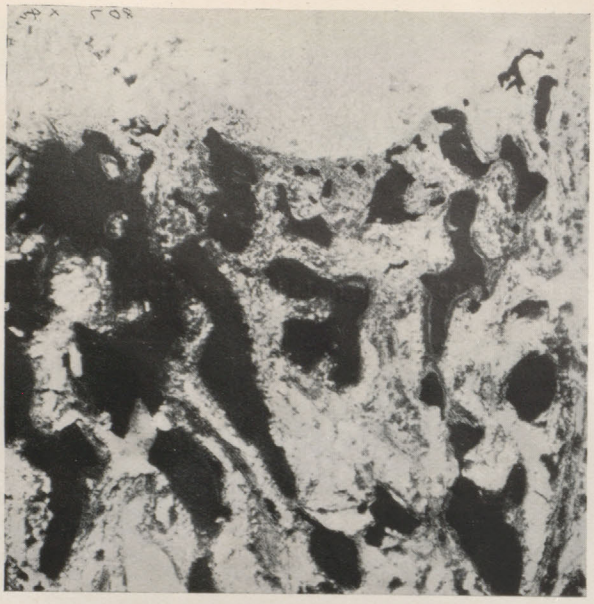


FIG. 93. Exp. 807. 60 per cent. white flour and 40 per cent. 'commercial wheat germ'. See also FIG. 90. Derangement of growth at junction.

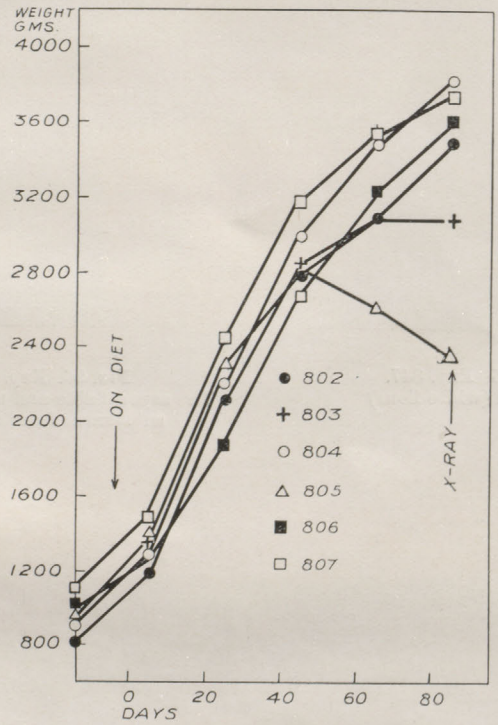


FIG. 94. Weight curves of 802, 803, 804, 805, 806, and 807. Note the falling off in weight of 805 after 7 weeks of diet owing to development of severe rickets.

FIGS. 95 and 96. Radiographs taken after 13 weeks of diet.

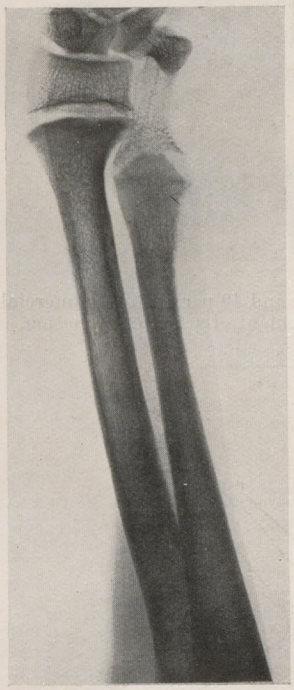


FIG. 95. Exp. 847.
Control (white flour)

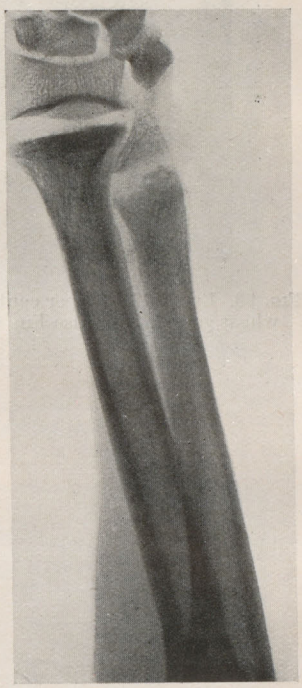


FIG. 96. Exp. 848.
The saponifiable and unsaponifiable substances from oatmeal.

FIGS. 97–101. Experiments 877, 878, 879, 880, 881. Radiographs taken after 6 weeks of diet. The diets of 877, 879, 880, and 881 were identical except for the exposure of some ingredients to radiations of mercury-vapour lamp.

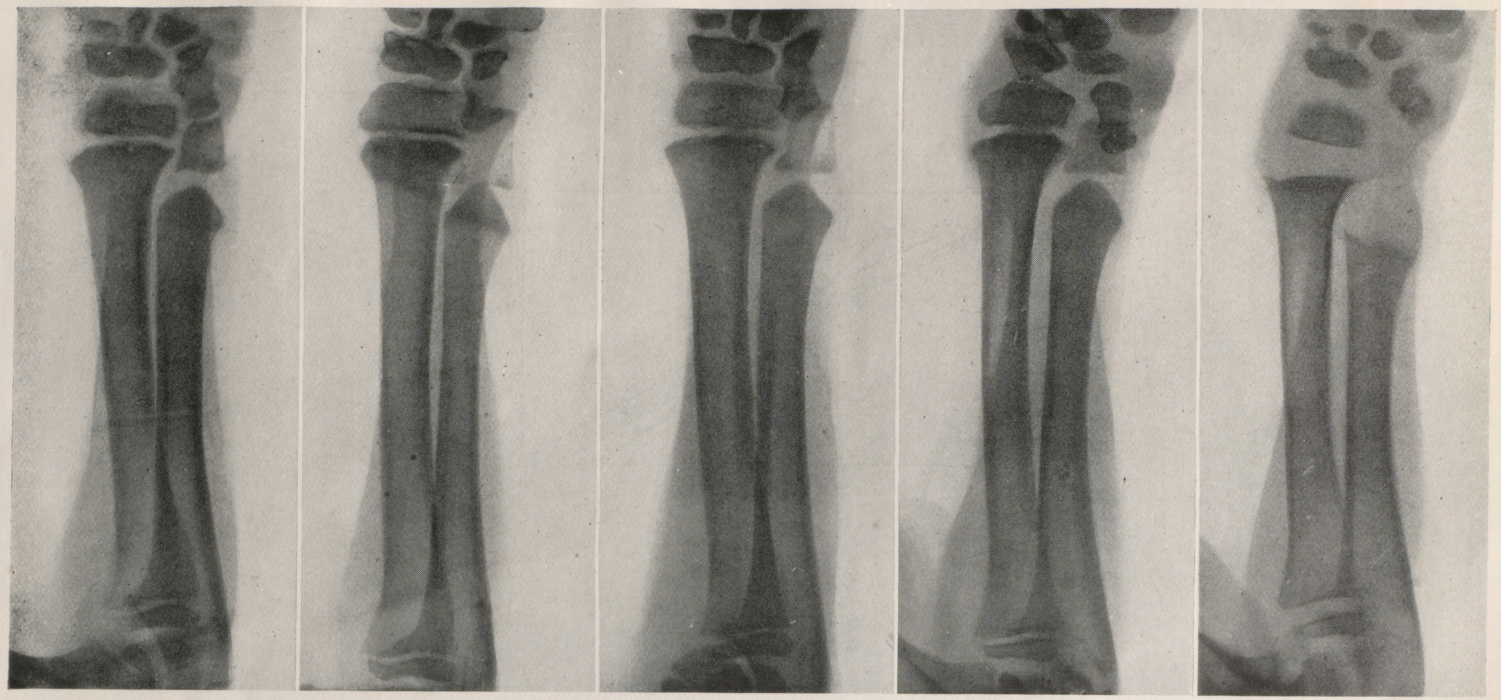


FIG. 97. Exp. 877. Oatmeal radiated by means of ultra-violet lamp.

FIG. 98. Exp. 878. Food not radiated. Diet contained 1 c.cm. of cod-liver oil replacing 1 c.cm. of olive oil.

FIG. 99. Exp. 879. Oatmeal cooked then radiated.

FIG. 100. Exp. 880. Olive oil radiated, oatmeal not radiated.

FIG. 101. Exp. 881. Food not radiated ; this is the only animal of the series with severe rickets.

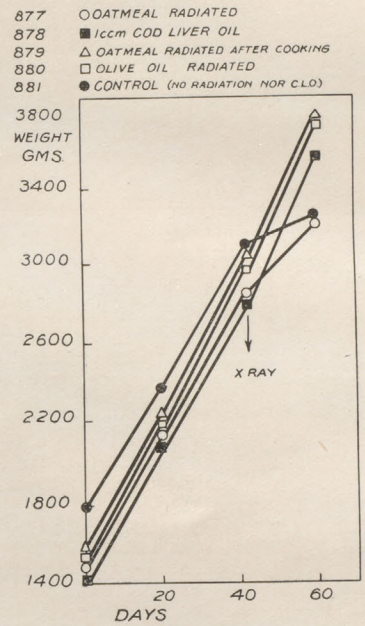


FIG. 102. Weight curves of 877, 878, 879, 880, and 881.

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