STUDIES ON EXPERIMENTAL RICKETS.

XXI. AN EXPERIMENTAL DEMONSTRATION OF THE EXISTENCE OF A VITAMIN WHICH PROMOTES CALCIUM DEPOSITION.

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With the discovery in 1913 that certain fats contain a substance or substances which are essential for growth, this class of foodstuffs assumed an importance in nutrition which had not been hitherto accorded it. At first the evidence of the existence of the substance which subsequently became known as fat-soluble A, or vitamin A, rested upon the failure of experimental animals to grow when the fats carrying this substance were lacking in the food, and the resumption of growtn when such fats were administered. Later xerophthalmia of a certain type was recognized as a pathological condition which invariably results from specific starvation for fat-soluble A. We have recently, however, convinced ourselves that a similar ophthalmia may be the result of disturbance of the balance of inorganic elements in the diet. Up to the present time no definite evidence has been brought forward to show whether one or more than one substance is contained in those fats which contain fat-soluble A which gives them their unique biological value (1). The great activity in several laboratories in the study of the cause or causes of rickets and related conditions has, during the last 3 years, brought to light the fact that there is a rôle played by fats in the etiology of this disease. This observation causes us to appreciate further the importance of the fat moiety of the food supply, and emphasizes the necessity

of providing in the diet a regular and abundant supply of such fats as promote the normal development of the skeletal tissues.

Mellanby (2) was the first to associate the group of fats which, because of their content of fat-soluble A were frequently distinguished as "growth-promoting" fats with the prevention of rickets. His work focused attention upon the problem as to whether fat-soluble A is itself a substance essential for the normal growth of bone.

Mellanby (3) was so impressed with the power of butter fat to protect puppies against the abnormalities of bone growth that he stated:

"These facts of agreement from the point of view of physiological reaction seem to me strong evidence that the substance in fats stimulating the calcification of bone is the same as Fat-soluble A, i.e. the factor which stimulates growth in rats."

He further says:

"Rats as regards growth and puppies as regards rickets become more independent of the vitamin as they grow older. These facts are in favor of Fat-soluble A being also responsible for the anti-rachitic action of certain fats."

All of Mellanby's own data cannot be brought into line with this reasoning. For example, Dog 187 was fed bread, yeast, salt, orange juice, separated milk, and 10 cc. of cottonseed oil, but no meat. It developed rickets. Dog 305 had the same diet but with 10 gm. of meat. The bones of this animal are described as "practically normal" (3). Meat is essentially lacking in fat-soluble A. It does, however, contain phosphorus, and but little calcium, and serves to change the ratio between these elements in the diet. This, as has been pointed out, is of great importance in the etiology of rickets (4).

In his experiments Mellanby studied cod liver oil, butter fat, lard, suet, bacon fat, peanut oil, olive oil, coconut oil, linseed oil, babassu oil, rape seed oil, palm kernel oil, and hydrogenated fats. He states (3):

"Of the fats tested, cod-liver oil is the best. Suet and butter also have a potent influence on calcification. Lard is poor as compared with suet. Butter, heated and oxidized for four hours, loses some anti-rachitic action.

Cod liver oil similarly treated still has a strong anti-rachitic effect. The vegetable oils vary in their anti-rachitic action, the order of merit being somewhat as follows: pea-nut and coco-nut oils (best), rape-seed, cotton-seed, palm-kernel, olive, linseed, and babassu oils (worst). Hydrogenated fats are poor."

It will be seen from the foregoing quotations that the question as to whether fat-soluble A exerts an antirachitic effect, or whether such action is to be referred to some other principle which has in many cases at least, a similar distribution is still open.

In July, 1921 (5) we pointed out on the basis of experimental data that

"We have not found it possible to demonstrate experimentally a difference between the effects of cod liver oil and butter fat when the content of the diet in calcium and phosphorus is near the optimum, the other elements in the diet being satisfactory."

We stated further that

"These results suggest the possibility that a dietary essential distinct from the anti-ophthalmic substance (fat-soluble A) may exist. If this is the case this would appear to be present in butter fat in small amounts, but to be very abundant in cod liver oil."

Again, in January, 1922 (6) we set forth evidence which was all but conclusive that there is a specific calcium-depositing substance. We stated

"The results of this series of experiments were so consistent and decisive that we can deduce no other conclusion than that cod liver oil contains in abundance some substance which is present in butter fat in but very slight amounts, and which exerts a directive influence on bone development and enables animals to develop with an inadequate supply of calcium much better than they could otherwise do. This substance is apparently distinct from fat-soluble A, which is essential for growth and which is associated definitely with the prevention of ophthalmia (keratomalacia)."

Our own experience had convinced us that existing methods were incapable of differentiating beyond doubt between fat-soluble A and a special calcium-depositing substance should such exist. We therefore formulated a plan which involved a comparison of a selected list of fats in respect to three kinds of effects in nutrition. First, we tested cod liver oil, shark liver oil, butter fat, and several vegetable oils for potency in causing the cure of xerophthalmia

due to lack of fat-soluble A. Secondly, we made comparative tests of the same fats to determine their value in promoting growth in young rats which were restricted to a diet so low in calcium that satisfactory growth was not possible without the provision of some substance which would make for a greater efficiency in the utilization of calcium than that which could be effected in its absence. Thirdly, we further studied these same fats by means of our "line test" to discover their relative values for inducing the deposition of the line of calcium salts in rachitic bones. With the data which we have secured from these three distinct types of tests, we are now in a position to interpret accurately the results of much of the experimental data in the literature which is otherwise confusing.

EXPERIMENTAL PROCEDURE.

I. Test for Fat-Soluble A.

The diet used for testing the value of different fats for the cure of xerophthalmia had the following formula.

Lot 3392.

	per cent
Rolled oats	40.0
Casein	5.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	52.5

This diet is essentially lacking in fat-soluble A. If 2 per cent of butter fat or cod liver oil is included in place of an equivalent amount of dextrin, xerophthalmia never develops, and the animals are able to grow.

In making the tests referred to in this paper young rats of 40 to 60 gm. weight were restricted to Diet 3392 until the puffiness of the eyelids was distinctly evident. At this point the eyes were frequently sealed shut while the rats slept, and were opened with difficulty on awakening. The malnutrition induced by this diet progresses rapidly to a fatal termination unless a suitable amount of fat-soluble A is provided at this stage. On the addition of sufficient fat containing fat-soluble A, at the time when the

edema of the eyelids is just becoming severe the swelling rapidly disappears, and the eyes return to a normal appearance within a few days. 2 per cent of cod liver oil, 3 per cent of shark liver oil, 3 per cent of burbot liver oil,[1] or 2 per cent of butter fat, were found to effect the prompt cure of incipient xerophthalmia under the conditions of our tests. Although we have data from other experiments which indicate that certain of the vegetable oils when fed liberally (8 to 20 per cent) from the beginning of the experiment tended to defer the onset of xerophthalmia, we have never found that such amounts of vegetable fats would cure the eye condition after it had once developed. It is, therefore, possible that there are traces of fat-soluble A in some vegetable fats. It should be specially noted here that 15 per cent of coconut oil did not cure or prevent xerophthalmia. We are convinced that a properly conducted *curative* test such as we have described is much more delicate than a *preventive* test can ever be made.

Hopkins (7) was the first to point out that oxidation destroys fat-soluble A. He showed that if oxygen is allowed to pass through heated butter fat the fat-soluble vitamin is readily destroyed. With this destruction the butter fat loses its power of inducing growth or of curing ophthalmia of dietary origin. Mellanby attempted (3) to make use of this means of destroying fat-soluble A in order to determine whether there is a distinct "anti-rachitic substance." He found butter fat of little value for protecting against rickets after it had been oxidized, whereas cod liver oil after the same treatment, *i.e.* heated to 120°C. for 4 hours while oxygen was passing through it, still protected his animals against rickets. He states:

> "If it should happen that four hours' heating and oxidation at 120°C. also leaves a large amount of Fat-soluble A in the cod liver oil, it will go a long way, especially when considered together with the butter results, to clinch completely the identity of fat-soluble A and the anti-rachitic vitamine."

Mellanby used no method of testing for fat-soluble A as distinct from the calcium-depositing substance since he did not make use of the ophthalmia test for fat-soluble A.

[1] We are indebted to Miss Ethel Kalmbach of Sturgeon Bay, Wis., who kindly furnished us a large sample of carefully prepared oil from the livers of the burbot.

We have found that cod liver oil treated with a stream of air bubbles at the temperature of boiling water for 12 to 20 hours no longer contains sufficient fat-soluble A to relieve rats from xerophthalmia when administered to the extent of 2 per cent of the diet. Cod liver oil which had been oxidized 4 hours, when fed as 2 per cent of the diet, cures xerophthalmia. Untreated cod liver oil under these conditions invariably causes complete recovery within 5 days. Likewise, 2 per cent of fresh butter fat, under exactly comparable experimental conditions, effects the disappearance of ophthalmia within 5 to 10 days. These results are sufficient to serve as a basis of comparison for our present purpose of the relative values of cod liver oil and butter fat for the cure of ophthalmia. The significance of these results in connection with the problem of the existence of a special calcium-depositing vitamin will be discussed later.

II. Tests of Fats for Their Protective Power against the Effects of Deficient Calcium Supply.

In a former paper (5) we have described experiments which showed clearly that a diet may be so deficient in calcium as to prevent growth in young rats. Butter fat failed to protect the animals from the effects of calcium deficiency, whereas the same diet supplemented with cod liver oil may promote good growth. We have since refined the technique of this type of experiment for studying the influence of fats on calcium metabolism. For this purpose we now use the following diet.

Diet 2947.

	per cent
Whole wheat	25.0
Whole maize	19.5
Rice polished	9.5
Rolled oats	9.5
Whole milk powder	5.0
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
Dextrin	1.5

The dietary properties of this food mixture have been carefully determined. The proteins are of very good quality and are abundant (about 23 per cent). Its content of water-soluble B is entirely adequate. It is somewhat deficient in fat-soluble A, but not sufficiently so as to induce xerophthalmia even if an animal were confined throughout life to it. Its inorganic content was such as to induce skeletal deformities. The diet was low in calcium, and not far from the optimum in phosphorus, and our problem was to see to what extent if any the different fats increase the efficiency of the animals in utilizing the small amount of calcium at their disposal.

This food mixture was fed with the following fats: cod liver oil 1.0 per cent; butter fat 10.0 per cent; shark liver oil 3.0 per cent; coconut oil 10.0 per cent; cottonseed oil 10.0 per cent; and olive oil 10.0 per cent.

An inspection of the charts shows that on this diet the animals cannot grow appreciably when the fat which is supplied them, is cottonseed or olive oil. When cod liver oil, shark liver oil, or butter fat is fed, growth proceeds in a fairly satisfactory manner. Coconut oil is the only one of the vegetable oils examined which increased the efficiency of the animals in utilizing their very low calcium supply. These data show clearly that there is a very remarkable property of certain fats which makes them of extraordinary importance in relation to calcium metabolism.

III. Tests of Fats for Their Power to Stimulate Healing in Rickets.

The data presented above relative to the values of several fats for relieving xerophthalmia, and for increasing the efficiency of the tissues in utilizing calcium when this element is present in very inadequate amounts in the diet, do not constitute a safe basis for deciding whether the effects in both types of experiments were due to fat-soluble A, or to two distinct substances. The results of the experiments in which coconut oil was studied (Chart 4, Lot 3008) suggest strongly that this oil contains a substance which improves the utilization of calcium. It has been pointed out above on the basis of experimental tests that coconut oil does not contain the substance, fat-soluble A, which

relieves xerophthalmia, since no improvement follows the ingestion of liberal amounts of this fat even in the incipient stages of the eye disease. Experiments directed toward preventing xerophthalmia by feeding coconut oil confirmed this view. We, therefore, supplemented the data obtained by the above described tests, with observations on the effects of the several fats on the initiation of the healing process in rickets. For this purpose we used the technique which we have called the "line test."

Diet No. 3143 employed in the "line test" consists of:

Diet 3143.

	per cent
Whole wheat kernel	33.0
Whole maize kernel	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

The details concerning the properties of this diet and its effects on the bones have been given in another publication (8). It is only necessary to state here that it contains a very inadequate amount of fat-soluble A, but sufficient to prevent xerophthalmia during the interval necessary to develop an exaggerated rickets and to observe the incipient healing of the lesion. Although the diet contains an *excessive* amount of calcium, no deposition of calcium salts takes place.

A few words of explanation are necessary regarding the column in Table I headed "Number of days in preparatory period." In order to carry out the "line test" satisfactorily the animals are fed Diet 3143 until a pathological metaphysis has developed satisfactorily. This state we have come to recognize by certain peculiarities in the movements of the young rats which we use as the test animals. The gait is unsteady and the hind quarters waver from side to side. When they move off rapidly they hop, usually favoring one hind leg. We have found it safe to rely on young rats which exhibit this abnormality of movement to show the histological picture which is essential for the conduct of the test.

We have examined histologically the bones of a large number of rats which have been kept on this diet for varying periods, and

TABLE I.

Kind of oil.	Per cent.	Number of days in preparatory period.	Number of days fat was given.	Number of animals.	Results.
Cod liver oil.	2	21	5	6	Healing rickets.
" " "	2	25	6	2	" "
" " "	2	28	11	3	" "
" " "	2	49	5	3	" "
" " "	2	49	5	3	" "
" " "	0.2	70	5	1	Severe rickets, a few specks of calcium in cartilage.
" " "	0.4	70	5	1	Severe rickets, no healing.
" " "	0.6	70	5	1	Beginning healing of rickets.
Cod liver oil (oxidized 4 hrs.)	2	25	6	3	Healing rickets.
Cod liver oil (oxidized 12 hrs.)	2	28	11	3	" "
Cod liver oil (oxidized 20 hrs.)	2	29	10	3	" "
Butter fat.	30	21	14	2	Beginning healing.
" "	30	32	11	4	" "
" "	15	17*	12	4	3 of these animals showed occasional specks of calcium in cartilage.
" "	15	20	20	1†	Beginning healing rickets.
Shark liver oil.	2	34	11	3	Marked healing.
Burbot liver oil.	2	30	6	2	Healing well on.
" " "	2	30	10	4	" " "
Coconut oil.	20	24	15	5	Very slight evidences of healing.
Maize oil.	20	34	15	5	Severe rickets, no healing.
Olive "	20	34	15	5	" " " "
Cottonseed oil.	20	29	14	5	" " " "
Sesame oil.	20	24	15	5	" " " "

* This preparatory period was too short. The metaphyses were narrow and irregular. It was possible to mistake calcified matrix which had existed before the onset of he disease as evidence of healing.

† This animal was badly deformed and feeble after being fed the butter fat for 20 days. There was no clinical evidence that the butter fat had been at all beneficial.

have found considerable difference in the length of time required to prepare them for the test for the calcium-depositing vitamin. We have gained the impression that it takes their bones a decidedly longer time in summer than in winter to deviate from the normal histology in the manner desired. Certainly they do not all respond in a certain number of days so that it is not possible to state accurately when they will be ready. We can, however, rely with certainty on the peculiarities of movement described above as a criterion of the time when a substance to be tested should be administered. The age of the animals is an important factor in the rate at which they may be prepared for the test. Rats weighing 55 to 60 gm. are as a rule, in fall, spring, and winter, ready in 28 to 35 days.

We have studied in this way the fats already discussed, and have included maize oil and sesame oil among the vegetable oils examined. Coconut oil was found to be distinctly more effective as a calcium-depositing agent than any of the other vegetables tested. It was, however, inferior to butter fat or any of the fish oils tested.

The several oils studied are listed in Table I. The amounts fed, the number of days of administration, the length of the preliminary period, the number of animals tested, and the results of the tests are given. Since starvation causes the deposition of calcium salts in the bones under the conditions of this test, we have invariably kept accurate records of food consumption in making these tests. The food consumption was adequate in all cases (9).

An inspection of Table I shows that cod liver oil, shark liver oil, and burbot liver oil, were highly effective in moderate doses in causing the deposition of calcium in the bones of rachitic animals. Butter fat is also effective when fed in large amounts (15 to 30 per cent), but it was necessary to extend the time of administration to 14 days in order to obtain even a faint calcification of the bones.

Coconut oil, when fed at 20 per cent of the diet, caused in 15 days the deposition of small amounts of calcium salts in the bones under the conditions of our test. Maize oil, olive oil, cottonseed oil, and sesame oil, were likewise fed at 20 per cent of the diet for 14 to 15 days, but in no case was there any tendency to the deposition of calcium salts.

Samples of cod liver oil which had been oxidized for 4, 12, and 20 hours, respectively, were tested for their calcium-depositing properties. Those oxidized 12 and 20 hours showed this potency in a degree apparently comparable with similar amounts of unoxidized samples, notwithstanding the fact that they had entirely lost their power to cure xerophthalmia. The samples which had been oxidized for 4 hours still cured xerophthalmia when 2 per cent of the diet consisted of the oil. They likewise gave a positive test for calcium-depositing power.

DISCUSSION OF RESULTS.

We have shown experimentally that cod liver oil oxidized for 12 to 20 hours does not cure xerophthalmia in rats. It does, however, cause the deposition of calcium in the bones of young rats which are suffering from rickets. This shows that oxidation destroys fat-soluble A without destroying another substance which plays an important rôle in bone growth.

Coconut oil is shown to be lacking in fat-soluble A, since it will neither prevent nor cure xerophthalmia. This oil, on the other hand, contains a substance which stimulates the deposition of calcium salts in rickets in a manner similar to cod liver oil. It is, like butter fat, far less effective from a quantitative standpoint.

Cod liver oil, shark liver oil, and burbot liver oil, are highly effective for curing xerophthalmia, for protecting the body against the effects of a deficiency of calcium, and for the deposition of lime salts in rachitic bones.

Certain vegetable fats, among which are cottonseed oil, maize oil, sesame oil, and olive oil, do not possess the property of curing xerophthalmia, nor do they raise the efficiency of the tissues in utilizing calcium when there is an inadequate provision, nor of initiating healing in rickets.

Butter fat contains the calcium-depositing factor but in much smaller amounts than the fish oils we have examined. It is a much better source of fat-soluble A than of the substance which regulates calcium metabolism.

Our results are in harmony with those of Mellanby in that they show that coconut oil has an antirachitic effect. They prove conclusively, however, that this effect is not due to the presence

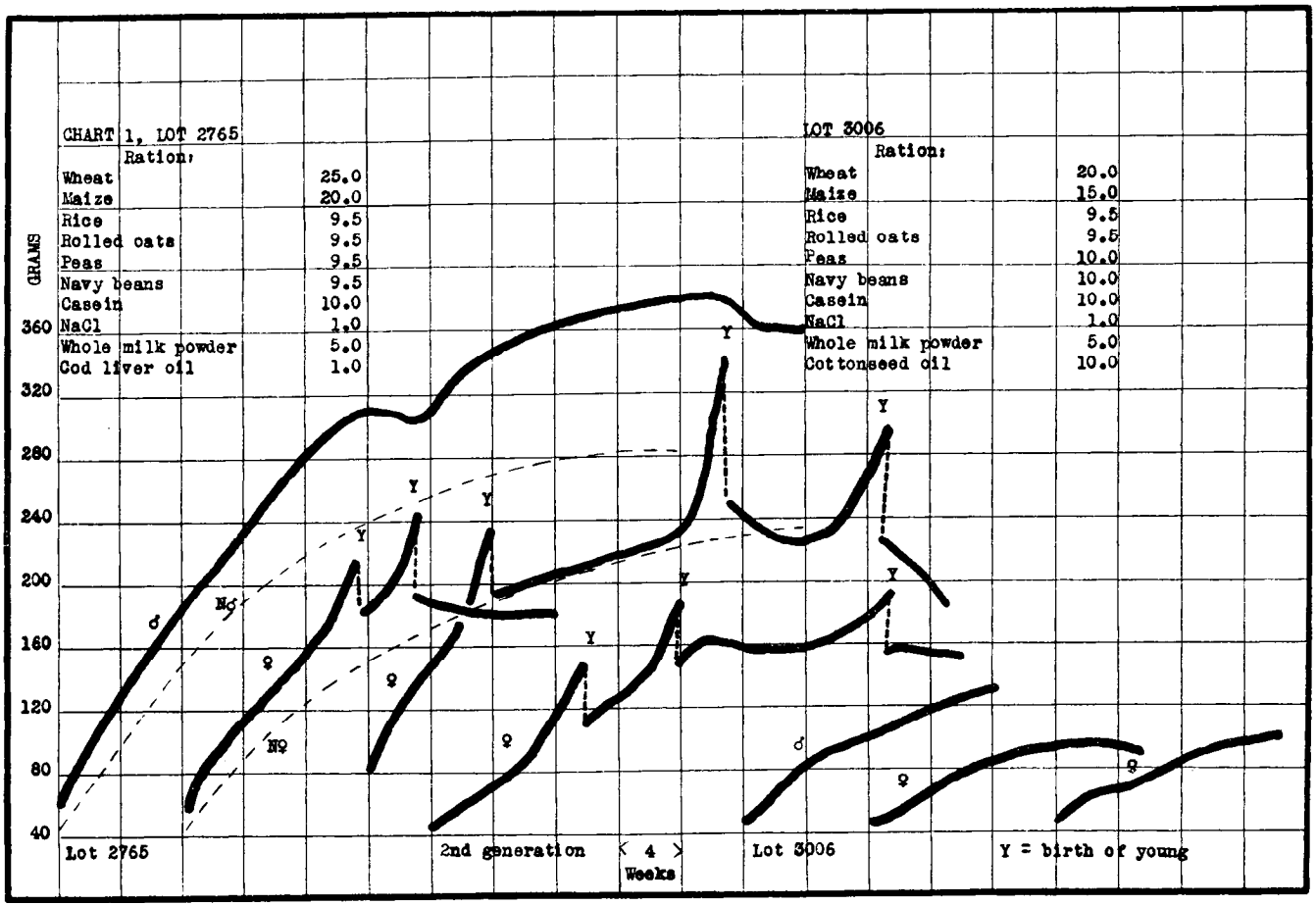
of fat-soluble A in this fat. Mellanby did not appreciate the importance of calcium and phosphorus in his experimental diets as a factor in the causation of rickets. Many of his apparently discordant results can, we believe, be accounted for on this basis rather than on the content of his diets in the calcium-depositing vitamin (3).

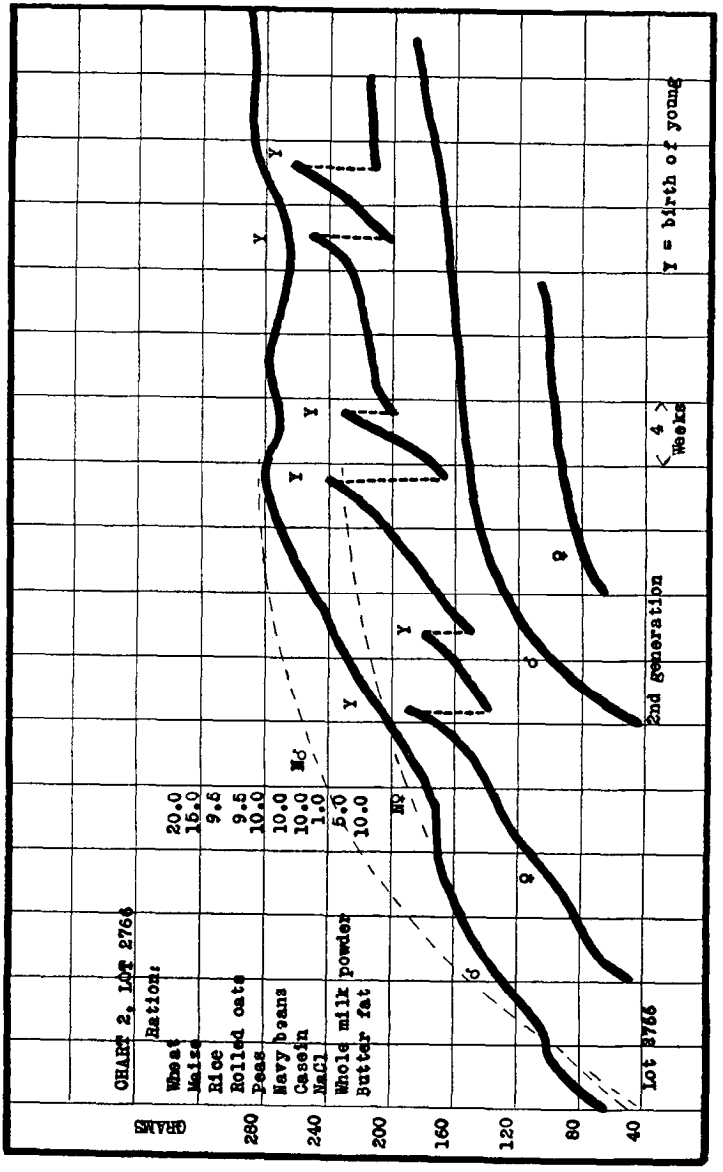
The question might be raised as to whether the effect of the substance which is designated fat-soluble A in protecting the functional activity of the eyes, is not one, and the calcium-depositing effect of certain oils which contain it, another physiological effect of one and the same substance. A controversy involving this principle has been carried on for many years over the identity or non-identity of pepsin and rennin. It may be postulated that the two properties which cod liver oil and certain other fats can be shown to possess, are referable to two side chains on the same molecule, and that in oxidation we have destroyed one and left the other intact. It is, of course, not possible at present to prove or disprove either of these views. The only evidence which bears on the question is the observation that coconut oil which had received no chemical treatment whatever, has been shown to possess demonstrable calcium-depositing properties, whereas it does not show a comparable antixerophthalmic effect. This points to the two properties under discussion being due to distinct substances.

The evidence set forth in this paper demonstrates that the power of certain fats to initiate the healing of rickets depends on the presence in them of a substance which is distinct from fat-soluble A. These experiments clearly demonstrate the existence of a fourth vitamin whose specific property, as far as we can tell at present, is to regulate the metabolism of the bones.

CHART 1. Lots 2765 and 3006 were fed diets which were essentially identical in their dietary properties except in so far as these were modified by the added fats. Diet 2765 contained 1.0 per cent of cod liver oil and Diet 3006 contained 10.0 per cent of a bleached cottonseed oil (Wesson oil) The defects in these diets were limited, as far as we can definitely characterize them at present, to a deficiency in calcium which was closely comparable in the two diets, and in such organic factors as certain oils (*e.g.* cod liver oil or butter fat) can supply.

The difference in the well being of these two groups of rats, due entirely to the qualities of the fats which they were fed, was most remarkable and





are illustrated by the curves in the chart. The group receiving the cod liver oil grew well and were fairly fertile and succeeded, notwithstanding the lack of calcium, in rearing most of their young. The group fed cottonseed oil, although they consumed about ten times as much fat, grew but little and failed early without having any young.

Lot 2765 received a suitable amount of both fat-soluble A and of the calcium-depositing vitamin, whereas Lot 3006 was essentially deprived of the calcium-depositing vitamin and secured but an inadequate amount of fat-soluble A in the seed products and milk powder which the diet contained. Lot 2765 was capable, because of the character of their diet, of utilizing effectively the small amount of calcium at their disposal, whereas Lot 3006 could not do this.

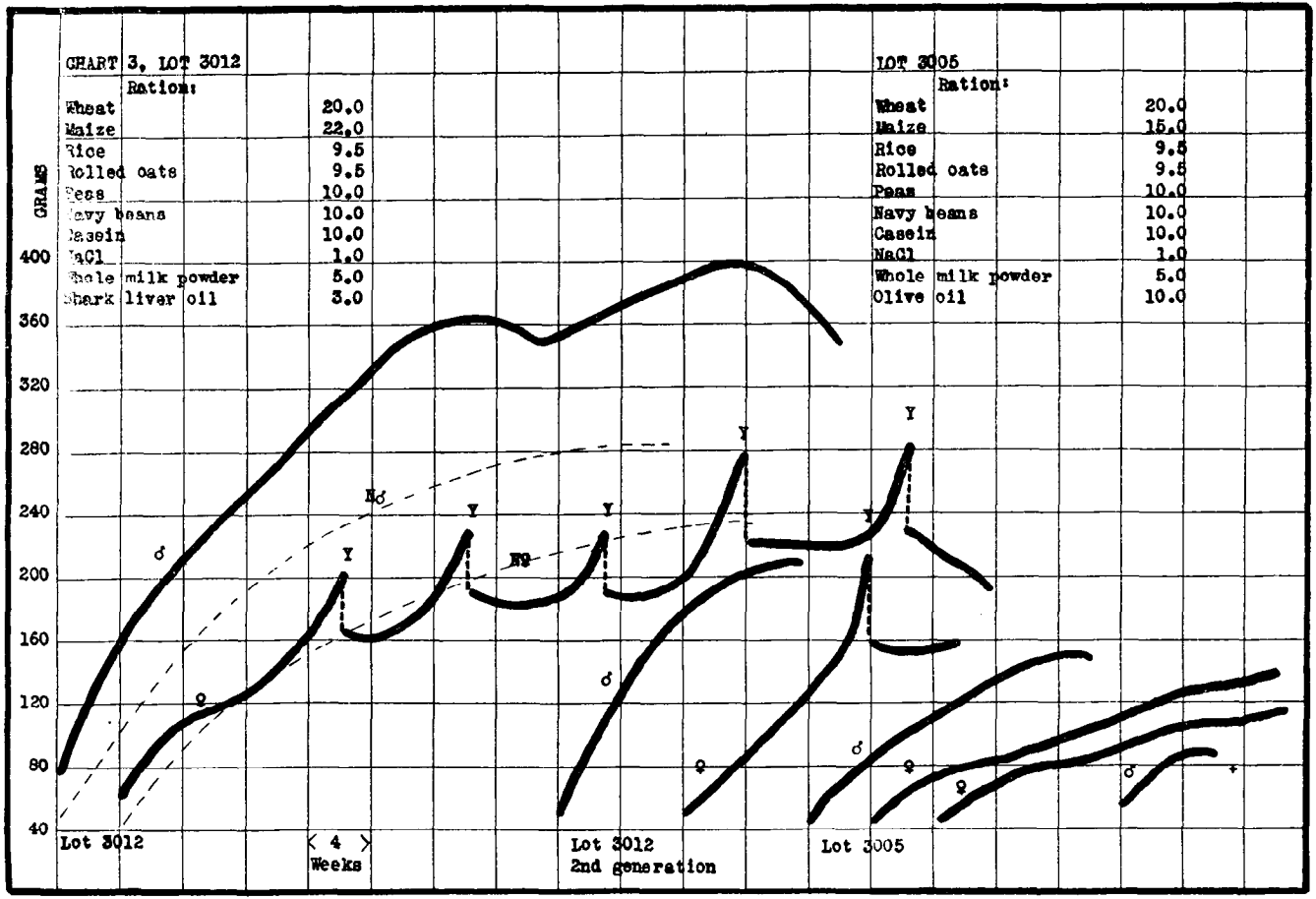
CHART 2. Lot 2766 had a diet like those described in Chart 1, but with 10 per cent of butter fat instead of cod liver oil or cottonseed oil. These animals grew fairly well but were decidedly inferior to those described in Chart 1, which received 1 per cent of cod liver oil. These records emphasize how very important it is to have in the diet certain fats possessing unique properties, when there is an unfavorable concentration of calcium in the food. Certain fats greatly protect the cells against their faulty chemical environment and enable them to utilize better than would otherwise be possible their very inadequate calcium supply. This, we are now in a position to assert, is due to the content in such fats of a special calcium-depositing vitamin which is often associated with fat-soluble A, but is distinct from it.

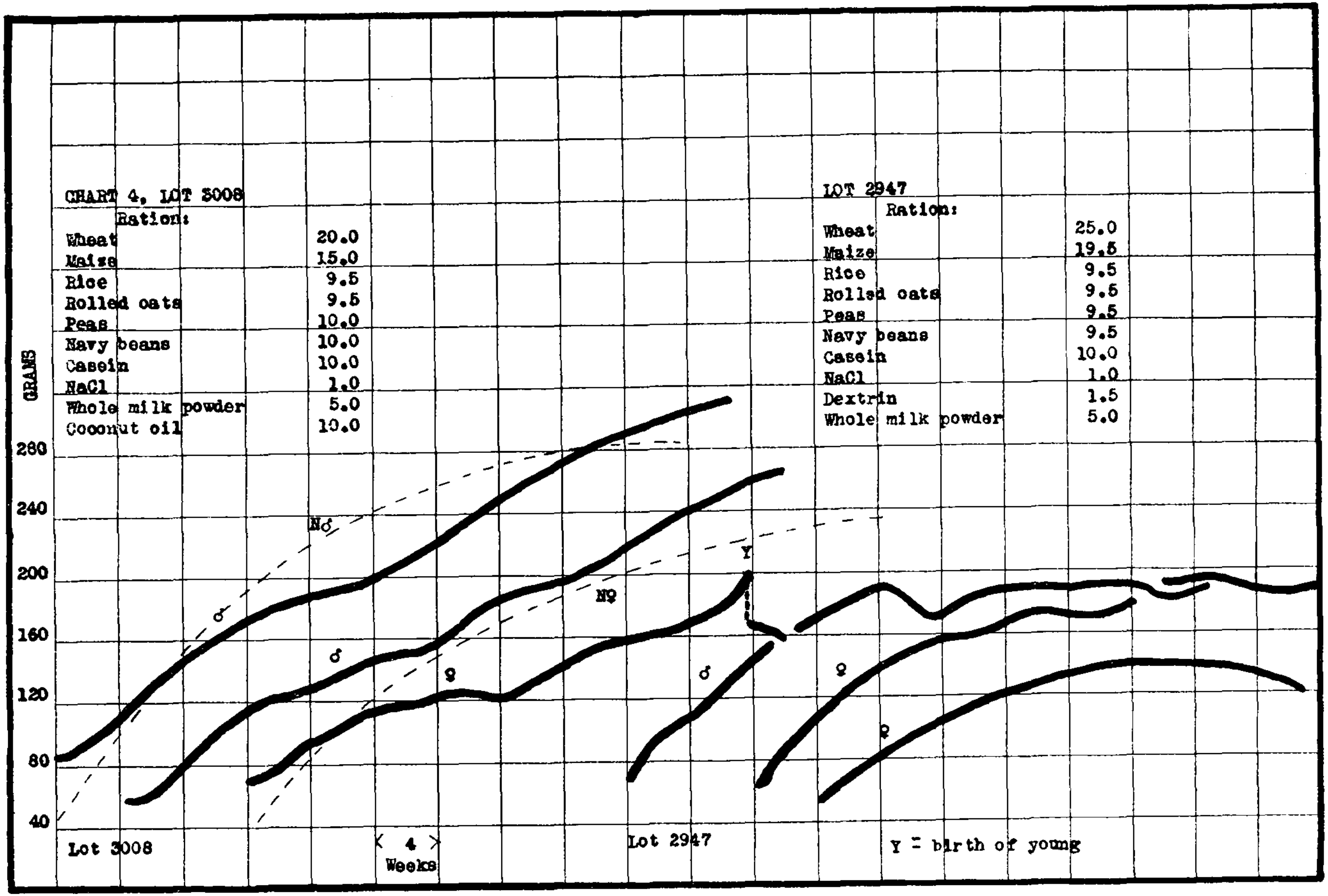
CHART 3. Lots 3012 and 3005 had the same food mixture discussed in Charts 1 and 2 except that the former had 3.0 per cent of shark liver oil and the latter 10 per cent of virgin olive oil. The contrast between the value of the fish oil on the one hand and the vegetable oil on the other in protecting the animals against the detrimental effects of a deficiency of calcium in the diet is very striking. Lot 3012 had a sufficient amount of both fat-soluble A and of the calcium-depositing vitamin. Lot 3005 had a suboptimal amount of the former, but very little of the latter, derived from the small amount of milk in its diet. The shark liver oil was not so effective as cod liver oil in raising the potential of the body cells so as to make them capable of utilizing an insufficient calcium supply, but it was distinctly better than butter fat for this purpose.

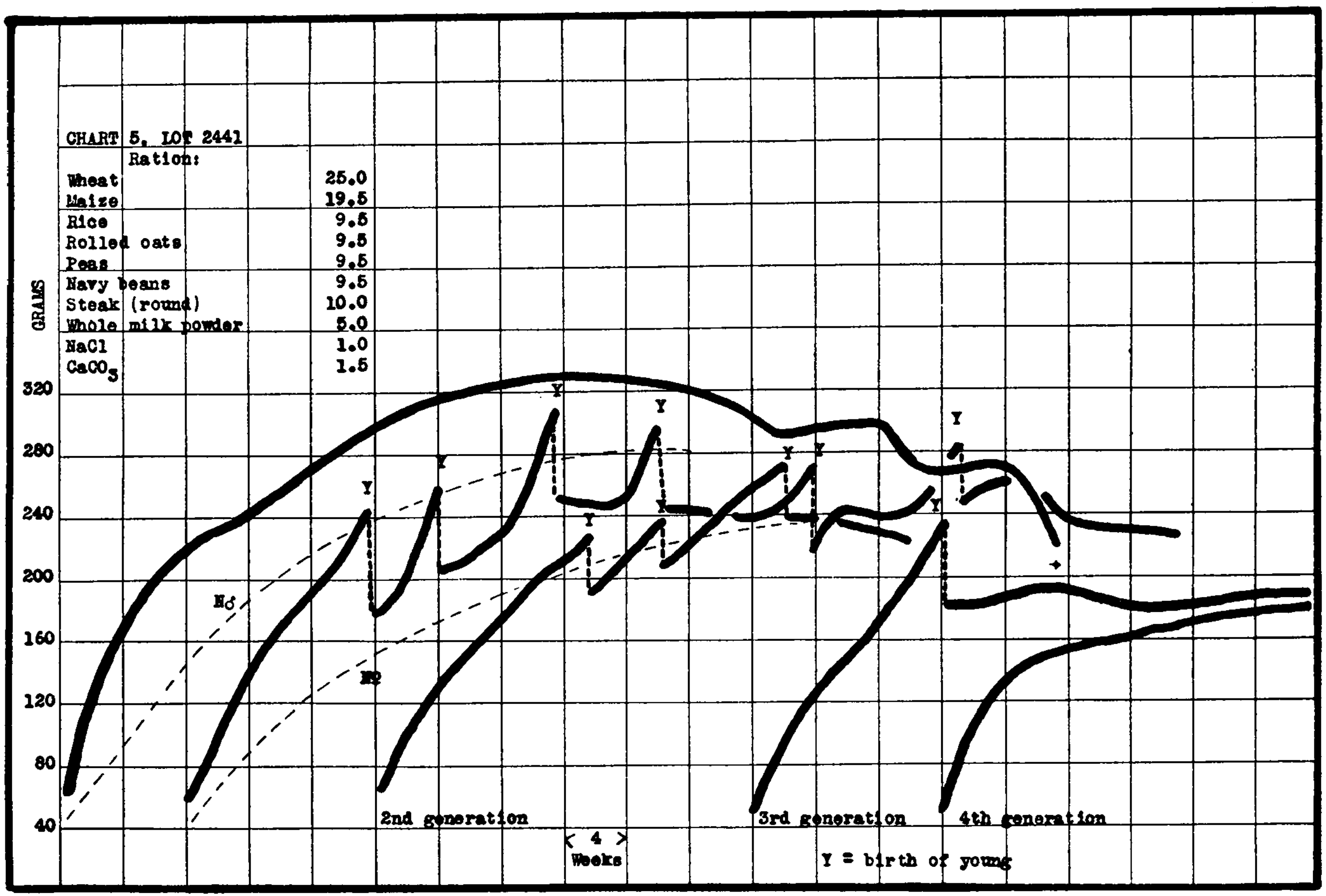
CHART 4. This chart is of special importance when its two groups of animals, Lots 3008 and 2947, are compared, and also these with Lot 3005, Chart 3, and Lot 3006, Chart 1.

Lot 2947 had the experimental diet previously discussed but without any added fats. On this they were able to grow but poorly, remaining undersized and infertile, and they deteriorated at an age when, on a better diet, they would still have been in possession of full vigor.

Lot 3008 is of special interest when compared with Lot 2947 which had the same diet without added fats and Lot 3005 (Chart 3) which had 10 per







cent of olive oil, and Lot 3006, Chart 1, which had 10 per cent of cottonseed oil.

One striking feature about this series of curves is that Lot 3005 (Chart 3) was not quite so well nourished on its diet containing olive oil as was Lot 2947 which had the same diet with no fats added. This difference was not sufficiently pronounced to establish it as significant further than to show that olive oil certainly does not have the property of increasing the efficiency of the cells in functioning with an inadequate calcium supply.

The diet consumed by Lots 3005 and 2947 was the same as that of Lot 3006 (Chart 1) but modified in that cottonseed oil was included to the extent of 10 per cent. A comparison of these groups shows that the basal diet itself did not raise the efficiency of the cells in utilizing their insufficient calcium supply. Cottonseed oil and olive oil lack, therefore, a property which is possessed in high degree by cod liver oil, shark liver oil, burbot liver oil, and to a lesser extent, by butter fat. These fats of animal origin differ from the two plant oils mentioned in that the former exercise a pronounced protective action in directing the functioning of the anatomic elements of the osseous tissues, whereas the latter do not.

Of special interest is the record of Lot 3008, whose diet was the same as those just discussed except that it contained 10 per cent of coconut oil. With this diet there was an observable protective effect due to the coconut oil. There is, therefore, a slight difference between olive oil and cottonseed oil on the one hand, and coconut oil on the other, with respect to the special effect on the bone growth. The animals on this diet containing coconut oil were not so much protected by that oil as their growth curves would seem to indicate. They were very short bodied and stocky creatures with rough coats and presented rather a miserable appearance notwithstanding the fact that they grew at a slow rate to about two-thirds the normal adult size.

If we employ this diet without the coconut oil or other added fat and add 1.5 per cent of calcium carbonate, the animals thrive far better than they do with the calcium omitted and coconut oil included. See Chart 5.

It should be kept in mind in interpreting these records that the diets are not very faulty in any respect other than in a lack of calcium. The small content of milk powder containing some butter fat, and the small amount of fat-soluble A furnished by wheat and maize, gave the animals nearly enough of this principle to meet their minimal requirements. The butter fat contained in the milk powder supplemented the very feeble protective power of the coconut oil in respect to its effects on the bones due to the calcium-depositing vitamin. Any fat possessing in considerable degree the special property of increasing the efficiency of the cells in utilizing a low calcium supply would have exercised a much more marked beneficial effect on the general well being of the animals than did the coconut oil.

CHART 5. The records in this chart illustrate the fact that animals can undergo apparently normal development when furnished a very small amount of the calcium-depositing vitamin, provided the diet contains

somewhere near the optimal content of calcium and phosphorus. The adjustment of the calcium content of these diets at a very low level has enabled us to demonstrate the difference in the quality of fats in respect to their content of the calcium-depositing vitamin in a manner which would have been impossible if the diets had contained a more favorable concentration of calcium and phosphorus.

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