FAT-SOLUBLE VITAMINE.

VII. THE FAT-SOLUBLE VITAMINE AND YELLOW PIGMENTATION IN ANIMAL FATS WITH SOME OBSERVATIONS ON ITS STABILITY TO SAPONIFICATION.*

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When in the early part of 1919, we (1, 2) formulated the working hypotheses, in our fat-soluble vitamine investigation, that the vitamine might be identical with, or closely related to certain yellow pigments of the carotinoid type, we made the attempt to correlate its occurrence on this basis, to formulate a procedure for its isolation, and to collect information as to its possible chemical nature. In these objects we have been substantially aided by our hypotheses, for while all the possibilities anticipated have not materialized yet there has been given a direction to our experimental efforts in this field, and apparently to those of others, which has lead to great centralization of effort. It was obvious that, in spite of the numerous instances of association of the physiological growth-promoting property which is attributed to the presence of fat-soluble vitamine and yellow plant pigments, the two would not necessarily have to be identical, in fact there need be no material relationship in composition or structure, as their coincident occurrence in nature might be due to physiological determination, pure and simple. In this event, with the diversification of metabolic processes which obtain in the plant and animal kingdom, it was to be expected that sooner or later the fat-soluble vitamine would be found to be present in a menstruum entirely free from pigments of the carotinoid type. To run across such an instance, appears to

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have been the good fortune of Palmer and Kempster (3), who demonstrated that pork liver, rich in the vitamine, contained no pigments of the aforementioned character. Gross of this laboratory has obtained similar results but Rosenheim and Drummond (4) purport to have shown that while carotin and xanthophyll are absent, another pigment of this type is present. It may still be said that in such instances where the fat-soluble vitamine is found to occur in the absence of color the pigment is present in the leuco form (2). While this can be accepted as a possibility it is at present not worthy of serious consideration because nothing is known of the structure of the carotinoid molecules and therefore nothing is known as to the probability of the existence of leuco compounds in the series. Nevertheless, as far as studies in this domain have been pursued, both in regard to distribution of vitamine and pigment and in regard to their physical and chemical properties, there is left no doubt but that chemically and physiologically they are related. These relations will be discussed in succeeding papers in which our results will be correlated with those of others who have been active in this field of investigation. In this paper it is desired to present data on the fat-soluble vitamine content of various animal fats as correlated with yellow pigmentation, and to record a few observations made on its stability to saponification.

EXPERIMENTAL.

For the laboratory technique employed in these experiments the reader is referred to previous papers of this series (5–10), as the experimental methods employed were essentially the same. In no instance has it been deemed permissible to make any selection of data. Four animals, usually two males and two females, were started in each group and continued until death or until the differences among the groups became so pronounced as to make further continuation superfluous. In a few instances where data were purely confirmatory we have felt at liberty to present merely representative illustrations.

Failure of growth alone has not been accepted by us as a good criterion to use in establishing the lack of the fat-soluble vitamine

\(^1\) Unpublished data.
and this for very obvious reasons. Emphasis was, therefore, always placed upon the appearance of the eyes of the rats, as an inflamed condition, an ophthalmia variously termed a conjunctivitis, a xerophthalmia, and a keratomalacia, makes its appearance in the vast majority of animals on a fat-soluble vitamin-Poor diet as first pointed out by Osborne and Mendel (11). Though the exact condition which is incident when the eyes become infected is not described accurately by these terms we have used them rather indiscriminately in the past hoping that pathologists would soon describe the condition in detail and designate it accurately. Stephenson and Clark (12) introduced the term keratomalacia in preference to xerophthalmia, apparently because in these inflammatory reactions a softening of the cornea with loss of the lens frequently results. In a certain sense each of these terms has its proper application in the various types or stages of the ophthalmitis observed. Generally a conjunctivitis with an erythema and edema of the eyelids appears first, later a keratitis or inflammation of the cornea results, and ultimately, if the diet is not corrected or if resistance to the infection does not develop of its own accord especially in severely purulent inflammatory reactions, a dryness or xerosis of the eye—a xerophthalmia—marks the culmination of the eye symptoms. As usually observed and especially in the primary stages the condition is marked by an excessive secretion and is certainly not a xerophthalmia. We believe that, in most instances when the inflammation is not confined to the conjunctiva, the infection is most accurately designated as a keratoconjunctivitis. Only occasionally will we have reason to speak of a xerophthalmia because true xerophthalmia has rarely made its appearance in our colony. When the inflammatory reaction is so severe as to cause permanent injury and the diet is not corrected, death results rapidly; if it is transitory, making its appearance now and then—indicative of only a partial deficiency of the fat-soluble vitamin—then practically complete recovery has always been observed. The recovery cannot be considered entirely complete because an inflamed condition of

2 Such studies have recently been published by Wason (Wason, I. M., J. Am. Med. Assn., 1921, lxxvi, 908), since the preparation of this manuscript.
the eyes, beyond a mere infiltration of fluid—an edema of the conjunctiva and eyelids—leaves its permanent mark in the destruction of the hair follicles on the lids. This "bare eyed" condition always arouses our suspicions of a fat-soluble vitamin deficiency, suggesting that a transitory reaction has escaped our notice if the inflammation was not actually observed.

In addition to the inflammation of the eyes, we have observed in many animals, large and small, an apparent resistance to infection of the eyes even though continually exposed by contact to severely infected animals, all on a fat-soluble vitamin-free ration. In such individuals an enophthalmia or a "small eyed condition" is frequently observed. The eyeballs are not "beaded" as in normal rats but appear small and sunken in the orbital cavity. Many of these individuals succumb to respiratory infections, in fact we have been led to think that possibly a certain immunity to infection of the eyes is thus conferred.

The incidence of respiratory infections as part of the syndrome induced by fat-soluble vitamin deficiency was described by McCollum (13) in his early work. It may consist, as we have observed, of a nasal or bronchial catarrh or even pulmonary infection with mucous or purulent exudate, at times even resulting in hemorrhage. Animals thus afflicted in the early stages of the disease sneeze and cough violently but later as the inflammation becomes confined more to the lungs the cough subsides and dyspnea becomes very pronounced with the slightest activity. Such animals fail very rapidly and even with the introduction of fat-soluble vitamin in the ration rarely show normal growth subsequently.

A fat-soluble vitamin deficiency is also far from being conducive to normal cutaneous nutrition so that very often—especially after an age of 4 months has been reached—evidence of dermal malnutrition makes its appearance. The fur appears bushy and thin, cutaneous growths occur on the tail, ears, and nose, and finally sores, which heal with difficulty, appear on the feet, limbs, and body; all bear testimony to this state of malnutrition.

All of the aforementioned symptoms and conditions have been carefully watched and noted at the time of the weekly weighings of our animals unless their condition or the nature of the
experiment made examinations at shorter time intervals of moment. Judgment was based on the sum total of indications.

_Fat-Soluble Vitamine Content of Cod Liver Oil._

Since Osborne and Mendel (14) in their pioneering studies on the fat-soluble vitamine found cod liver oil to contain this dietary essential surprisingly little experimental endeavor has been made to study it in its quantitative relations though repeated emphasis has been placed on the efficacy with which it can be used as a therapeutic agent in perverted metabolism of the osteoid tissues. As far as known to the writers, Zilva and Miura (15) are the only investigators who have studied this problem. They have recently published a preliminary note on their experiments stating that cod liver oil in its crude state was found to be 250 times as potent as butter in furnishing this constituent.

Our investigations were not outlined to bring out such extreme differences as we were primarily intent on a correlation of the fat-soluble vitamine with pigment content in comparison with the intense pigmentation of butter, and selected cod liver oil because we had at hand an excellent sample which in comparison with butter was practically devoid of yellow pigments—it had only a faint yellowish green color. If the vitamine were a yellow pigment it should therefore have shown only limited activity. The sample of oil was prepared from fresh cod livers with minimum heat exposure. The livers were cut into small pieces, put into a steam-jacketed open cooker, and heated not to exceed 180° F. The oil collecting on the surface was skimmed off as rapidly as it formed in the first 35 minutes of heating, filtered through paper, and bottled.

As seen in Chart 1, this oil when fed at a level corresponding to 2 and 5 gm. of material in a kilo of ration was exceedingly active; it was far more efficient than any sample of butter fat that we have ever studied. This alone was one of the things which made us very skeptical in the early course of our studies of the assumption that the fat-soluble vitamine was necessarily a yellow pigment.
Seasonal Variation in the Fat-Soluble Vitamine Content of Butter.

Much has been written and said about the fat-soluble vitamine content of butter fat primarily because of its extensive use as an article of food and therefore its probable importance as a source of the fat-soluble vitamine in the human dietary. Due to the comparatively low intake of butter fat in the diet of the adult, the destruction of its vitamine in some cooking processes, the general occurrence of the vitamine in many foods such as green plant tissue, certain seeds and even roots, and the undetermined requirements of man, it is questionable whether its vitamine content would now attract the attention that it does if it were not for the fact that butter fat was the material in which the vitamine was first discovered. Early in our studies we were impressed with the fact that butters varied decidedly in their vitamine content; most of them being very rich in this dietary constituent, but some being as poor as the average oleomargarine (5). Certain observations which we made also impressed us with the lability of the vitamine as we found it destroyed in heated butters and in butters kept under poor storage conditions (5), so that we did not feel at liberty to draw conclusions with respect to the primary causal factors involved.

As our studies on the distribution of the fat-soluble vitamine progressed and indications of the occurrence of the vitamine with yellow pigments were obtained it appeared profitable to attempt to correlate these relations in butter fat especially in view of the fact, as is well known in dairy practice, that butter churned in late winter or early spring under Wisconsin conditions without artificial coloring is practically void of all color. If the vitamine content should be demonstrated to be of the same order of magnitude as the pigmentation then further presumptive evidence of this relation would have been obtained.

To this end there were prepared samples of butter fat in the latter part of the months of March, April, May, and June from cream obtained at the University creamery which was representative of the composite collection from a large number of dairy farms in the vicinity. The butter was churned in the laboratory, melted, and filtered at a low temperature, and then stored in a refrigerator till used in the experiments. The butter
fat was fed in basal rations which have been repeatedly demonstrated to be very low in the fat-soluble vitamine content, though controls were never omitted. Originally, through the March series of experiments, the basal ration was one which we have used before, consisting of: casein, 18; agar, 2; Salts 32, 4; ether-extracted wheat embryo, 6; and dextrin, 70. In the April, May, and June series a white corn ration, consisting of: white corn, 40; casein, 14; Salts 32, 3; Salts 35, 1; and dextrin, 42; was used. This latter is an excellent ration and guarantees a sufficiency of the water-soluble vitamine being introduced with the white corn which is not always the case when a variable commercial product such as wheat embryo is used as its source. The butter fats of unknown value were introduced in these rations at the expense of so much dextrin.

As illustrated in Charts 2, 3, and 4 there occurs a decided variation in the vitamine content of the different butters; but even gross inspection of the monthly collections made it evident to us that the variations in vitamine were not quantitatively reconcilable with the variations in pigment. To enable more accurate comparisons to be made we availed ourselves of the use of a standard color solution in a Duboscq colorimeter, which was prepared by dissolving 7 gm. of $\text{K}_2\text{Cr}_2\text{O}_7$ and 0.074 gm. of $\text{K}_2\text{Cr}_2\text{O}_7$ in water and making it up to 100 cc. volume. Such a solution compares favorably with the color of June butter fat but as the intensity of pigmentation is reduced, as is the result in the winter butter fat, the effect of a residual yellowish green pigmentation becomes disturbing and comparisons are not so easily made. Nevertheless, the accuracy of the determinations exceeded by far the requirements of our work as the results of the feeding trials themselves cannot be evaluated with any great degree of accuracy. With June butter fat accepted as having a value of 100, May butter fat was found to have a value of 86, and March and April butter fats, a value of 2.8. The latter were therefore practically colorless. With these factors in mind, upon inspection of the growth curves, it becomes increasingly evident that the fat-soluble vitamine content of the butter fats does not run parallel to the intensity of pigmentation; otherwise in the first place May and June butter fats fed at the 0.5 per cent levels should have been far more potent than they actually were.
in comparison with the March or April butter fats as they carried from 30 to 35 times as much pigment. In the second place when fed at different levels, 2 per cent for the March and April butter fats as compared with 0.5 per cent for the May and June butter fats, the former should not have exceeded the latter in efficiency as even then only from one-eighth to one-ninth as much total pigment had been introduced into the ration. These findings harmonize with those of Drummond and Coward (16) who arrived at similar conclusions.

Before the facts of these relations were obtained many attempts were made in the summer of 1919 to ascertain if any parallelism between vitamine and pigment content obtained by taking advantage of the fact that the carotin in butter fat is easily destroyed by heating. We heated butter fat in deep and in shallow dishes in the presence and absence of oleic acid—as acids accelerate pigment destruction in butter very markedly—with and without aeration with hydrogen, carbon dioxide, and air. We expected that if vitamine and pigment were not identical under some of these conditions destruction of the one without destruction of the other might be found to occur. Our results were entirely unsatisfactory as consistent duplication of results on different samples could not be obtained. As the selection of data bearing out a particular point at issue is not justifiable when unexplainable contradictory evidence is also obtained, the results of this work were not published. They served to convince us, however, that the success of such experimental attempts depended largely upon good fortune as butter fat is too variable in fat-soluble vitamine content to be taken as a good source of vitamine for studies of this character.

Since these experiments were carried out, Stephenson (17) has submitted data which tend to show that charcoal can be successfully employed in the removal or destruction of the pigment without causing complete destruction of the vitamine. Unfortunately her experimental period is shorter than desirable, especially as she worked with animals of considerable size in which we have found under normal conditions the vitamine reserve to be high and continued normal growth for 8 weeks to be common. The sudden death of one individual is not reassuring as pulmonary infections carry off some individuals on
a fat-soluble vitamin-poor diet without premonitory symptoms in the course of a few days; nevertheless her data are very suggestive especially in view of Palmer's (3) observations on the feeding of pork liver.

Fat-Soluble Vitamin in Beet Fats.

Osborne and Mendel (18) and later Halliburton and Drummond (19) showed that beef fats might contain considerable amounts of the fat-soluble vitamin though in general their efficiency in furnishing this dietary constituent was not to be compared with butter fat. By fractionally crystallizing the beef fats from alcohol, Osborne and Mendel obtained a very active fraction. The beef oils were found to be exceedingly active while the solid residue was inactive. In our work a somewhat similar product, the oleo oils from beef fats, prepared in commerce for the manufacture of oleomargarine, were in some instances found richer in the fat-soluble vitamin than many butters (5). Subsequent to the publication of these results we became aware of the fact that the vitamin content as determined in our feeding experiments with these samples seemed to vary directly with the intensity of pigmentation. This led to the collection of additional data to determine if this was a mere coincidence or if it was commonly true.

During 1919 the experiments were confined to the investigation of the perinephric fat of animals of the Jersey, Durham, and Holstein breeds. The fatty tissue was ground in a meat hasher and extracted by heating slightly above the melting point in a steam oven and then straining and decanting the melted fats. They were preserved in Mason jars in a refrigerator until utilized in the experiments.

As seen in Chart 5, the Jersey fat was very active while the Durham fat gave no evidence of containing this vitamin. The same inactivity was shown by the Holstein fat. Both the Holstein and Durham fats were practically colorless; the Jersey fat, on the other hand was fully as pigmented as a sample of June butter.

In 1920 we duplicated these experiments except for the fact that the samples were not taken from any particular breeds
but were selected promiscuously from slaughtered animals for color intensity. The dark beef fat was fully equal in color to June butter, the medium beef fat was two-thirds as colored and the light beef fat only one-tenth as colored. These values were obtained by measurement in a Duboscq colorimeter. The results shown in Charts 6 and 7 are essentially of the same character as those obtained the year before—the fat-soluble vitamine content roughly parallels the pigmentation. In view of the results that we have obtained with butter fat, it is not to be concluded that this is necessarily always the case. The rapidity of fat deposition, its mobilization, and the variation in the assimilation of pigment with different breeds and individuals, no doubt all operate to modify the primary determinative effect of the composition of the ration. Just how the latter may influence the relations we have again had occasion to observe with the fat-soluble vitamine content of egg yolks. Normally, on ordinary rations light-colored yolks are low in the fat-soluble vitamine; yet by the selection of a special and unusual ration we have succeeded in producing light-colored yolks of normal vitamine content.

**Stability of Fat-Soluble Vitamine to Saponification.**

The study of the characteristics of the fat-soluble vitamine has presented considerable difficulties particularly due to mistaken notions of its stability and solubility properties which were fostered by suggestions rather than conclusive evidence as presented by various investigators. McCollum and Davis (20) reported the transference of the fat-soluble vitamine from butter fat into olive oil after the butter fat had been submitted to a mild saponification at room temperature. This was submitted as a preliminary paper in 1914 as it was stated that other experiments were under way and would be reported as soon as advisable. In the experiments detailed by them a number of difficulties can be appreciated which have made duplication very difficult as no confirmation of these attempts has been published. In the first place the drying of the soaps and the dissipation of the ether vapors from the ether-olive oil extract are processes not easily carried out under laboratory conditions without causing the destruction of considerable amounts of the vit-
amine. In the second place the olive oil extract as fed at a 3 per cent level and, therefore, equivalent to 6 per cent butter fat, did not leave a sufficient margin of fat-soluble vitamine to guarantee its presence at the close of operations as many samples of butter fat without having been subjected to any treatment are ineffective when fed at this level. In the experiments mentioned, however, it is possible that the 20 per cent lactose (21) carried considerable vitamine so that but a small increment was needed to elicit a growth response. Nevertheless, all these facts made it appear very unprofitable to attempt to repeat these experiments especially as the vitamine was ultimately brought into the solution of a fat with no determination of the completeness of the saponification beyond an inspection of the solubility of the reaction mixture which in the presence of so much soap is far from satisfactory. These experiments are therefore to be considered merely as a demonstration of the resistance of the fat-soluble vitamine to the mild saponification employed. In the light of this it was not surprising that Drummond (22) failed in demonstrating the resistance of the vitamine to the mild saponification of Henriqueus used by McCollum. He varied his procedure in that he attempted an ether extraction of the soaps but failed to show any activity of either the extract or an ether extract of the saponified residue when he fed the equivalent of 15 and 20 per cent of butter fat and whale oil, respectively.

From our work on the extraction of the vitamine from plant materials, where we adopted the method of separation in use for the carotinoids, we have demonstrated repeatedly the resistance of the vitamine to saponification and its subsequent extractibility by ether (10). From our present work (Chart 8), it is evident that the fat-soluble vitamine as found in animal fats has similar properties. In two instances, Lots 741 and 969, the saponification was conducted at 37°C. for 4 hours; 300 gm. of the fat being treated with 600 cc. of 20 per cent alcoholic potash, which are the proportions of fat and alkali used in the methods of analysis of The Association of Official Agricultural Chemists (23). At the end of the 4 hour period, 2,400 cc. of water were added and the aqueous alcoholic solution of soaps extracted three times with ether. The ether extracts were washed with a small volume of water and then evaporated
directly at room temperature in an air current on the ration. For control purposes a saponification was carried out with butter fat parallel to one run by the Official Methods using the same concentration and excess of alkali, acting for the same period of time at the same temperature as the preparation. They gave the same saponification value indicating that the saponification in our butter fat preparation was complete. Nevertheless, we made another preparation in which the fat was boiled with 20 per cent alcoholic potash for one-half hour as required by the Official Methods but with no reduction in alkali concentration. The growth curves of Lot 972 bear testimony to the fact that even under these drastic conditions the fat-soluble vitamine was not destroyed to any appreciable extent. From this it can be concluded that it is not a fat or an ester and that it is not labile to heat in the presence of a high concentration of alkali.

We desire to express our appreciation to Lord Brothers who furnished the cod liver oil and Armour and Company who furnished us with the beef fats.

SUMMARY.

In cod liver oil there is present a very high concentration of the fat-soluble vitamine with but small amounts of yellow pigments.

Butter fat shows a seasonal variation in the fat-soluble vitamine content when obtained from stall fed cows during the winter and pastured in the summer as is the practice under Wisconsin conditions.

The fat-soluble vitamine content of butter fat does not run closely parallel to the yellow pigment; yet in general, due to determination by their content in the feed, butters highly pigmented are rich in the vitamine; butters low in pigment should be looked upon with suspicion.

In beef fats the relations are somewhat similar; those most pigmented are also generally richest in their fat-soluble vitamine content.

The fat-soluble vitamine withstands severe methods of saponification. This indicates that it is not a fat and probably
not an ester and makes possible the compounding of satisfactory fat-free synthetic rations for investigative purposes.

BIBLIOGRAPHY.

The fat-soluble vitamin in cod liver oil

Chart 1. Lots 1072 and 1073 illustrate the remarkable growth-promoting property of small amounts of cod liver oil as a source of the fat-soluble vitamin. Rats 4285 and 4281 both continued their phenomenal rate of growth beyond the curves of growth shown here, the former weighing 395 gm. and the latter, 422 gm. 6 weeks later. Rat 4282 raised three young out of a litter of twelve in 5 weeks to an average weight of 40 gm. None of the other litters was raised. White Indian corn at a 40 per cent intake level was used as the source of water-soluble vitamin as it has been shown in numerous experiments to lead to nutritive failure as a source of the fat-soluble vitamin and yet it furnishes plenty of the water-soluble vitamin for normal growth.
CHART 2. This chart shows the variable effects in growth responses when an attempt is made to introduce the fat-soluble vitamin into the ration by the substitution of 0.5 per cent of butter fat collected in successive months for 0.5 per cent of dextrin. On the March collection in Lot 982 by the pronounced failure of growth and even maintenance in all the animals, by the dermal infections of Rats 3921, 3922, and 3923 and by the ophthalmia in Rats 3922, 3923, and 3924, there is left no doubt that a deficiency of the fat-soluble vitamin obtained. On the April collection, Lot 983, growth was considerably better but all the animals were afflicted with keratoconjunctivitis. On the May butter fat, Lot 985, growth was continuous but dermal nutrition was poor; no eye symptoms were observed. On June butter fat, Lot 1032, the experimental results were practically the same except that dermal malnutrition was not evident, and Rat 4122 contracted a prolonged bronchial infection. Young were not reared.
CHART 3. When the butter fat was increased to 1 per cent of the ration, growth was in all cases much improved with marked evidence of inferiority of the March product as fed in Lot 890. All of the rats contracted keratoconjunctivitis before their death and Rat 3553 by its sneezing and coughing indicated the presence of a respiratory infection. The April sample, although not up to par, was evidently richer in fat-soluble vitamine content, as the growth performance of the animals was much better and no indisputable symptoms of conjunctivitis were observed. There were, however, some indications of cutaneous malnutrition as the tails of the animals bore some infections towards the close of the experimental period. For May and June, Lots 986 and 1033, no special comments as to the normality of growth appear to be called for. On the May product Rat 3940 even raised a litter of four without apparent difficulty.
CHART 4. With the butter fat increased to 2 per cent of the ration deficiencies in the nutritive value of the collections, as far as evident were entirely eliminated, if we except the fact that Rat 3559 failed due to a localized caseous pulmonary infection. All other individuals remained normal.
This chart illustrates the growth performance of rats on the perinephric fats of cattle as a source of the fat-soluble vitamine. In Lot 902, Rats 3602 and 3604 were afflicted with keratoconjunctivitis, the eyes of the others being normal when the change to Ration B carrying the Jersey fat was made. Except for Rat 3603 the condition of the animals was poor so that the response to the improvement of the diet was probably not of the order of magnitude to be expected. This is suggested by the results shown by Lot 947.

Lot 905. When the rats of this lot were changed to the fat-containing ration, all of the rats were affected with ophthalmia. The eyes of only Rat 3617 showed improvement subsequent to the change before death ultimately supervened. This was the only suggestion of the possible presence of the fat-soluble vitamine; possible because improvement of eyes is sometimes though not generally observed without any change having been made in the diet, yet growth in such cases is not restored and death slowly results.

Lot 947 when started out on a ration containing ten parts of Durham fat, such as was substituted in the ration of Lot 905, did not enable the rats to grow any longer or better than when the fat was not included. All of the rats showed inflamed eye conditions which promptly subsided shortly after the change to Jersey fat was made. This was true even in the case of Rat 3784 which however, due to its impoverished condition, died shortly thereafter.
YELLOW PIGMENTS AND THE FAT-SOLUBLE VITAMINE IN BEEF FATS

CHART 6. This chart shows the growth observed on the 1920 beef fat samples when the animals were given the fat addition from the beginning of the experiment. In Lot 1052, on the light beef fat samples, Rat 4201 developed pulmonary infections and Rats 4203 and 4204, keratoconjunctivitis. The appearance of Rat 4202 alone remained fairly normal but rapid failure ensued after parturition which is very often observed on a fat-soluble vitamine-poor ration. In Lot 1053 on the medium beef fat Rat 4205 contracted conjunctivitis after 16 weeks on the ration, later by the 20th week its eyes turned purulent with complete recovery by the 26th week, but by that time indications of dermal malnutrition were very distinct; it showed loss of hair, localized infections on body, and a horny epithelial growth on its nose. Rats 4208 and 4207 gave indications of dermal malnutrition only. These conditions in general suggest a deficiency in the fat soluble vitamine content even though growth was fairly good. In Lot 1054 on the dark beef fat all the animals maintained themselves in good condition to the end of the experimental period.

107
Chart 7. Chart 7 illustrates the relative efficiency of the same beef fats illustrated in Chart 6 as determined by their ability to induce recovery in rats which had given indisputable evidence of a fat-soluble vitamine deficiency in the ration.

In Lot 992, Rats 3961 and 3963 had edematous eyes, respectively, at the end of the 6th and 7th weeks on the white corn ration. In the case of the former, temporary improvement was noted from time to time; in the latter, permanent improvement extended over a period of 6 weeks, but in neither case was growth resumed. This illustrates, what we have often observed, that in the vast majority of cases less of the vitamine is required to maintain normal eye conditions than to maintain growth. In Rats 3962 and 3964 the eyes were inflamed severely at the time of discontinuation of the trial.

In Lot 993, on the medium beef fat, Rat 3967 indicated incipient inflammation with slight edema of the conjunctiva which was promptly cured upon change of ration. Rats 3966 and 3968 also showed some indications of an edema; in the former it persisted in spite of change of ration.

On the dark beef fat in Lot 1017 previous to the change all rats except Rat 4064 showed a severe ophthalmitis. Rat 4064 showed an enopthalmia. In all cases where the change of ration was made improvement was prompt and recovery complete.
Stability of the Fat-Soluble Vitamine to Saponification

Chart 8. This chart shows the resistance of the fat-soluble vitamine to destruction by saponification of the fats in cod liver oil and butter fat as indicated by the prompt recovery of growth in the rats when an ether extract of a solution of the soaps was added to the basal rations. This consisted, in Lot 741, of a ration which had served in an experimental series to determine the fat-soluble vitamine content of butter fat. It is noteworthy that 0.7 per cent of this sample of July butter fat furnished no appreciable amounts of the vitamine. In Lots 969 and 972 our usual white corn ration was used. In no case, previous or subsequent to the change of ration, was any abnormality of eye conditions observed.