LXVII. RESEARCHES ON VITAMIN A. VII. NOTES ON THE FACTORS INFLUENCING THE VALUE OF MILK AND BUTTER AS SOURCES OF VITAMIN A.

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DURING the last few years we have had occasion to examine a large number of samples of butter and milk for the presence of vitamin A, and from the examination of this material, drawn from many sources and representing a wide variety of products, we have obtained results which throw some light on the factors governing the distribution of the vitamin in these important foodstuffs. At the same time we have made a number of controlled experiments to gain more definite information on one or two points which have arisen during the course of the work. In the present paper we have put together what we regard as the most important results we have obtained, in spite of the fact that the whole does not form a connected survey.

QUANTITATIVE METHOD OF TESTING MILK AND BUTTER FOR VITAMIN A.

The essential details of the method we have employed throughout this work have been described in a previous paper [Drummond and Coward, 1920, 1]. The procedure of giving the foodstuff to be tested as a small weighed or measured supplement to the experimental animals before they receive their daily ration of the basal diet is the only means of ensuring a quantitative intake, and should always be employed. By using this technique and by care in the selection of the test animals results may be obtained which are sufficiently accurate for the approximate estimation of the relative values of samples of milks or butters.

The most serious error in these experiments appears to be that introduced by the individual variation in the response of the test animals. This source of inaccuracy can never be entirely eliminated but it may be greatly reduced by care in their selection. In the routine testing of milks and butters it is our usual procedure to make the preliminary test by giving a group of animals whose growth has been satisfactorily inhibited by a diet deficient in vitamin A a daily supplement of 2 cc. or 0·2 g. respectively. There is seldom if ever any difficulty in making the animals eat the rations and the method is not nearly so troublesome as might at first appear. As soon as the day's supplement is consumed the animals are given their ration of the basal artificial food mixture. From the results of the preliminary test an indication of the subsequent procedure may be obtained. If good resumption of growth is obtained with 2 cc. of milk or 0·2 g. of butter, another group is tested with amounts of 1 cc. or 0·1 g. respectively, whilst if no growth is obtained on the first trial larger supplements are given until positive results are obtained. In this manner a series of curves showing recovery of growth at various rates may be obtained, and from a comparison of these rates a rough idea may be gained of the relative value of the foodstuffs being tested.

We would like to emphasise, as previous authors have done [*e.g.* Chick and Hume, 1919], the importance of knowing the actual intake of the foodstuffs being tested. The procedure followed by some investigators of incorporating the substance with the basal diet and of making no record of food consumption cannot yield results which are in any sense quantitative.

THE VITAMIN CONTENT OF MILK.

(*a*) *The influence of the diet of the mother.* It was originally shown by McCollum, Simmonds and Pitz [1916] and confirmed by Drummond [1918] that the milk secreted by the lactating female will tend to be deficient in vitamins unless her diet contains adequate amounts of these factors. More recently a great deal of experimental evidence has been obtained in support of this observation, and from our own observations we are inclined to believe that this cause is by far the most important in causing such variations.

Further reference to the influence of the diet on the food value of the milk, as illustrated by studies on vitamin A, will be made in the section on butter.

(*b*) *Influence of breed.* Our examination of milks has included a number of samples from cows of different breeds being fed on the same ration. These animals were all out on pasture which was at that time fresh and green. Unfortunately our results are not numerous enough nor do our observations extend over a period sufficiently long to enable us to arrive at a definite conclusion on the effect of breed on the nutritive value of the milk. The impression we gained, however, was that the milk obtained from cows of the Jersey and closely related breeds tended to be richer in vitamin A than that yielded by Shorthorns and Black Angus.

Much more work of a systematic nature is required to settle this point. It is interesting to recall in this connection the experiments of Palmer and his colleagues [1914] on the pigments of milk and butter. They showed that the yellow lipochrome pigments which naturally colour these foodstuffs are derived from the green fodder of the cow, and that they may be stored up in the body fat. The body fat of the Jersey breeds is much more deeply pigmented than that of most other types and they tend to yield a milk fat of a rich golden yellow colour. Now it has been shown that the storage of such yellow pigments in the body fat may in some species be accompanied by a storage of the vitamin A when the animals are on pasture [Rosenheim and Drummond, 1920; Drummond and Coward, 1920, 2], and it is therefore conceivable that the cows of the Jersey breeds have a higher storage capacity for vitamin A than others such as the Shorthorns, as they undoubtedly have a higher capacity for storing pigments. This might perhaps explain the higher vitamin A value of the milk yielded by grass-fed Jersey cattle, or it may on the other hand be connected with the high fat content characteristic of those milks.

(c) *Seasonal variation.* As far as we can ascertain there is no appreciable variation in the vitamin A content of milk at different seasons of the year, apart from that associated with the different character of the diet ordinarily consumed at those seasons. The amount of our information on this point is however very scanty.

The physiological significance of colostrum.

The theories to be found in the text-books of physiology at the present time regarding the function of colostrum scarcely appear to be adequate to explain why so highly specific a secretion should be provided during the first few days of the life of the offspring. It may be true that this product has a laxative action, to quote one of the existing theories, but one instinctively looks for a more fundamental function for this characteristic fluid. Whilst we do not claim to have discovered this function, we would like to point out that under normal conditions colostrum appears to be considerably richer in vitamin A than the later milk. Illustrations of this may be seen from the curves in Fig. 1 which compare the growth-stimulating power of the milks yielded by the same animals.

We have obtained this type of result on several occasions with various samples of colostrum and later milk, but with one lot of samples little appreciable difference could be detected.

The significance of our observation may be slight, but on the other hand it may indicate that the child during the first few days of life requires a relatively high concentration of certain substances, amongst them the vitamin A, either for its immediate requirements or to provide it with a good reserve at the outset. We are inclined to regard this higher value of colostrum as an indication of a mobilisation of the reserves of the mother, since it does not appear to be proportional to the fat content. The results show that the concentration of vitamin A in the fat of colostrum is decidedly higher than in the fat of the later milk.

It is again interesting to recall that there is also a partial mobilisation of the lipochrome pigments of the mother's body fat for the production of colostrum, which normally contains a much higher concentration of these colouring substances than the fat of the later milk.

At first our attention was directed to the high protein content of colostrum, since we were at that time considering the possibility of the vitamin A being of a lipoid character and attached to protein. We could not, however, obtain

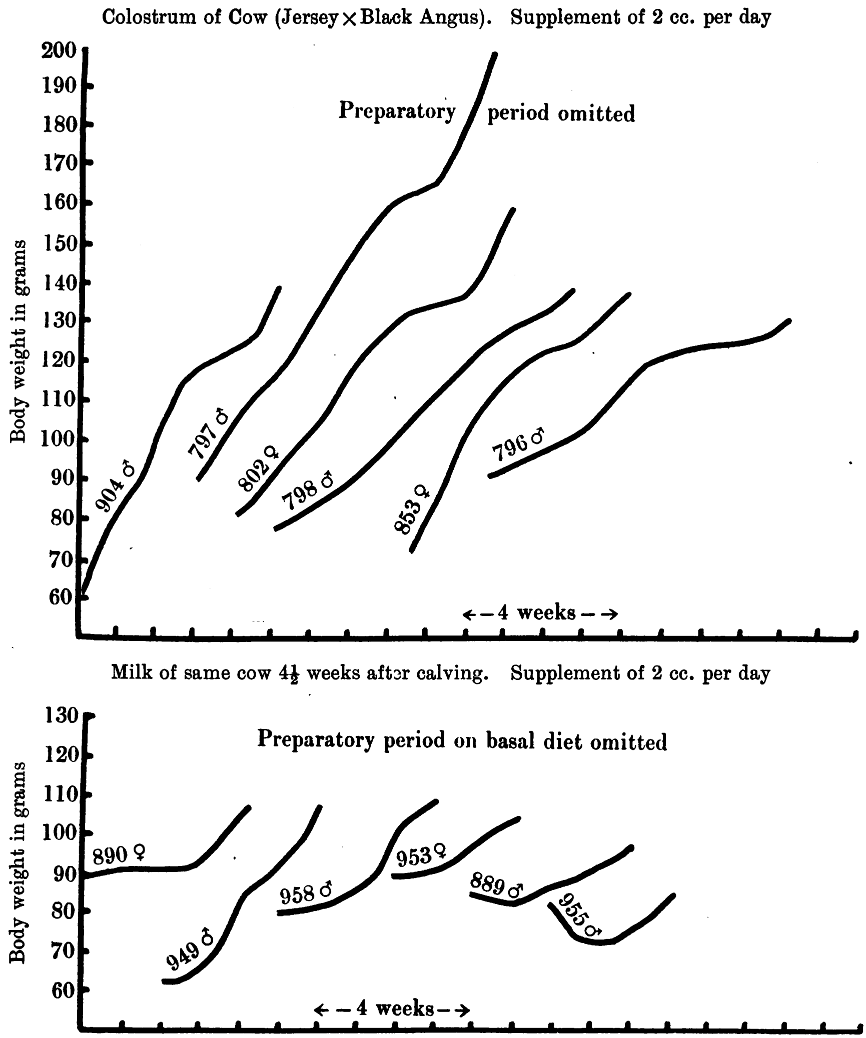

Fig. 1. Curves showing superior growth-promoting power of 2 cc. of colostrum per day to that of 2 cc. of later milk (lower curves).

any satisfactory evidence of the existence of such a protein-vitamin complex, and could not connect the high vitamin A value of colostrum with its high protein (other than caseinogen) content. From one experiment of somewhat short duration we learnt that there appears to be a gradual fall in the amount of vitamin A in the secretion as the colostrum is gradually replaced by the normal milk.

These observations may be of significance in practical nutrition, and should be considered in relation to the giving of colostrum to infants.

THE VITAMIN A CONTENT OF BUTTER.

(*a*) *The influence of butter-making.* Early in our investigations we were struck by the superior value of milk as a source of vitamin A over that possessed by the same quantity of milk fat in the form of butter. At first we were inclined to consider this in the light of the theory then occupying our attention that the vitamin exists in milk in the form of a complex with protein. The experimental facts have however led us to abandon this view and there appears little doubt that the loss of vitamin A in butter-making is partly mechanical and partly due to destruction.

As an example of the type of difference may be given the average figures we have obtained during our study of milks and butters. Fresh milk from cows fed on green pasture will in nearly all cases induce a resumption of growth in rats fed on a diet deficient in vitamin A when given in a daily supplement of from 2–3 cc. [cf. Hopkins, 1912 and 1920, 1]. This amount of milk may be regarded as supplying approximately 0·1 g. of fat per day. In order to induce the same amount of growth by adding a supplement of butter it is necessary to give about 0·2–0·3 g. per day.

In order to establish more definitely this relationship of milk and butter, a butter-making experiment was carried out through the kindness of Capt. J. Golding, D.S.O., at the Dairy of University College, Reading.

On June 2nd the morning milk of four shorthorn cows (first or second calf) was collected (7 gallons) and a sample of half a gallon immediately bottled and refrigerated for testing. The remainder was taken down to the college dairy, where, four hours after milking, it was separated in a centrifugal separator after pre-heating to 90° F. Four and a half pints of cream were obtained, and both cream and separated milk were cooled to 65° F. and placed in a cold store. Twenty-four hours later the cream was made into butter without having been submitted to any pasteurisation or artificial ripening process. The four and a half pints of cream were mixed with four and a half pints of water at 66° F., the mixture being at a temperature of about 52° F., and was churned in a 12-gallon churn of the ordinary type. Churning occupied half an hour and three pints of breaking water and a pint of rinsing water were used. Five quarts of buttermilk were obtained of which a sample was removed and refrigerated for testing. Two and a half gallons of water at 46° F. were added for washing, and, after draining through muslin, the butter was returned to the churn where it was salted by being turned a few times with $1\frac{1}{2}$ gallons of water containing 3 lbs. of salt. After draining the butter was worked and made up into a block. The four products, fresh milk, separated milk, buttermilk and butter, were kept in cold storage until used for testing on the rats.

The quantities used for testing were, original milk 2 cc., separated milk 2 cc., buttermilk 2 cc., butter 0·06 (the original milk contained 3·05 % fat) per day respectively.

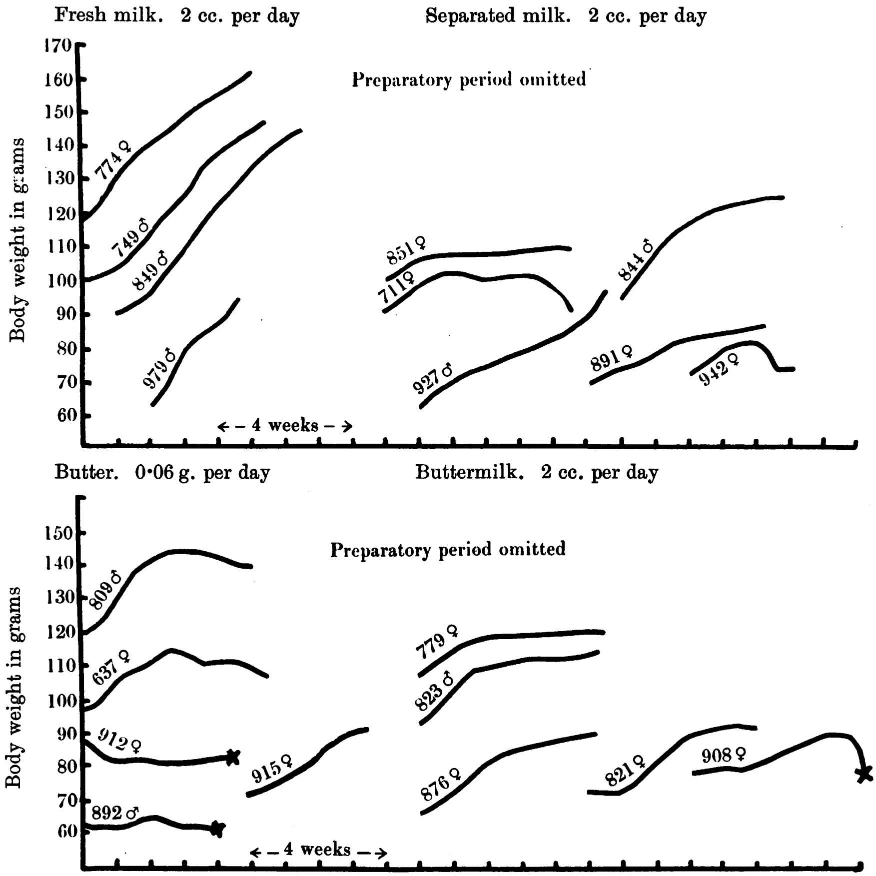

Fig. 2. Curves 774, 749, 849, and 979 show effect of 2 cc. original milk per day. The lower potency of the separated milk is shown in curves 851, 711, 927, 844, 891 and 942 and that of the buttermilk in 779, 823, 876, 821 and 908. The amount of butter equivalent to the whole milk gave much less growth (Curves 809, 637, 912, 915, 892).

As may be seen from the curves in Fig. 2 the rats fed on the supplement of 2 cc. per day of fresh milk resumed and maintained almost normal growth throughout the duration of the experiment. The rats to which approximately the corresponding amount of milk fat in the form of butter was given showed some growth (or none) for two weeks, when they ceased growth and declined. The rats fed on the supplement of separated milk (0·07 % fat) showed some growth, but in no case was it nearly so good as that given by the same amount of fresh milk, and later the growth curves became horizontal or in some cases declined.

The rats fed on the supplement of buttermilk showed growth very similar to that on the separated milk.

This controlled experiment confirms the opinion we had formed from the examination of a large number of miscellaneous samples that butter is usually a less potent source of vitamin A than the amount of milk which would yield that butter. The loss may apparently occur in more than one way. There is undoubtedly some loss by way of the separated milk which carries a small amount of fat containing vitamin A, but the possibility of loss occurring by chemical processes during the butter-making must also be considered.

It is difficult to estimate the chances of destruction during these processes, and although we know that the vitamin is readily inactivated by contact with air or oxygen, especially at high temperatures [Hopkins, 1920, 2; Drummond and Coward, 1920, 3; Zilva, 1920], the likelihood of such destruction having occurred in our experiment to any marked extent does not appear to be great. Possibly the large surface of the fat exposed to contact with air during churning may lead to some inactivation, since we know from the observations of Rogers [1909] that the amount of air entrapped in the butter during working, especially overworking, may cause oxidation of the non-fatty constituents. Although Hunziker [1920] does not entirely agree with the conclusions of Rogers he states that it is unquestionably true that any air permanently present in the butter becomes very finely divided and emulsified by overworking, and in this form a much larger surface area of the butter becomes exposed to the oxidative action of the air. Destruction of the vitamin by oxidative processes from this cause more probably occurs slowly during storage than in the short time occupied by the actual butter-making.

In much of the making of butter the cream undergoes several important processes which were not employed for the sample investigated above. The chief of these are pasteurisation and ripening, but the extent of the destruction during these processes cannot be judged until controlled experiments have been made with the various types of process commonly in use, and statements such as that of Hunziker [1920, p. 572] cannot be accepted until such experiments have been carried out. In this connection we may say that in examining average samples of London milk, all of which have been pasteurised by one process or another, we have encountered samples with a fairly high vitamin value, a finding that may perhaps indicate that in ordinary circumstances the loss by pasteurisation in milk at any rate is probably not serious. Daniels and Loughlin [1920] in a recent study of milks were unable to detect any loss of vitamin A by heat treatment, but a study of cream has not so far as we are aware been made. With regard to the effect of the ripening of cream for butter-making on the amount of vitamin present we have also no definite evidence. The process entails little contact with air and the temperature is kept low so that it is likely that little or no destruction of the factor occurs. On the other hand there is no justification for assuming as some have done that the growth of the organisms will increase the amount of vitamin, since we have evidence that this substance is not produced by the lower plants in the absence of chlorophyll [Coward and Drummond, 1921].

Reference to any standard work on the butter industry such as the valuable book by Hunziker [1920] shows that in some cases the cream is subjected to processes which must be suspected of causing more damage to the vitamin A than those we have already considered. These include the aeration or blowing of cream, a procedure which is believed by some to be of value in removing objectionable odours. Thus, for example, Ayres and Johnson [1914] demonstrated that certain undesirable flavours may be removed from cream and milk by blowing with a vigorous current of air at a temperature of 145–160° F. for a period of five minutes or more. The existing knowledge is sufficient to justify the conclusion that such a process would very materially lower the amount of vitamin A present.

In some cases considerable use is made of the process of neutralising acid cream to render it fit for butter-making.

It appears probable as Hunziker points out that the process of neutralisation of cream will cause little if any loss of vitamin A.

(*b*) *Butter as a source of vitamin A*. The early work in the study of the vitamins led to the belief that butter is a very rich source of vitamin A, and in a recent compilation of the knowledge of these substances [Medical Research Council, 1919] it was placed high in the list of foodstuffs. Since that list was compiled considerable advances have been made, and we now appreciate the very great importance of two facts, namely, that samples of butter may show a wide variation in the amount of vitamin present, and that in comparison with some other fats butter is not to be regarded as possessing so high a potency as was at first believed. Appreciation of these two points may help to explain certain of the discrepancies which have been encountered by some workers, particularly by those who are studying the vitamins in their relation to practical infant feeding.

The use of the roughly quantitative method of testing which we have elaborated enables us to form an approximate estimate of the relative value of individual samples of butter, and some which we have investigated have possessed a growth-promoting power little or no better than the average refined vegetable oils, which are usually classified low down in the list of foodstuffs containing vitamin A. Such samples are in nearly every case derived from cows on dry feed during the cold weather or when fresh green pasture is not available. Further confirmation of the influence of the feed on the vitamin value of the butter which was pointed out in a previous communication by Drummond and Coward [1920, 2] is afforded by the curves in Fig. 3. These curves represent the testing of samples of butter from a farm herd of Shorthorns. The first sample was obtained at the end of April 1921 and was typical of the butter produced by the cows then fed in stall on hay, cake and roots, being a hard, friable and almost colourless product. As may be seen from the curves in Fig. 3 this sample when tested in 0·2 g. per day had practically no value as a source of the vitamin.

A sample of the butter from the mixed milk of the same herd was obtained after they had been put out to grass for one week, the grass being at that time fresh and green. This sample was more highly pigmented with lipochromes—none of the samples contained added pigment—and was distinctly softer than the first sample. The effect of the short period on grass in raising the amount of vitamin present can be seen from the curves in Fig. 3 and is also seen in the case of a third sample prepared after the animals had been out for nearly a month. As is well known this year has been remarkable for a most prolonged and severe drought which has gradually dried up and withered the pasture land to a degree seldom seen in this country.

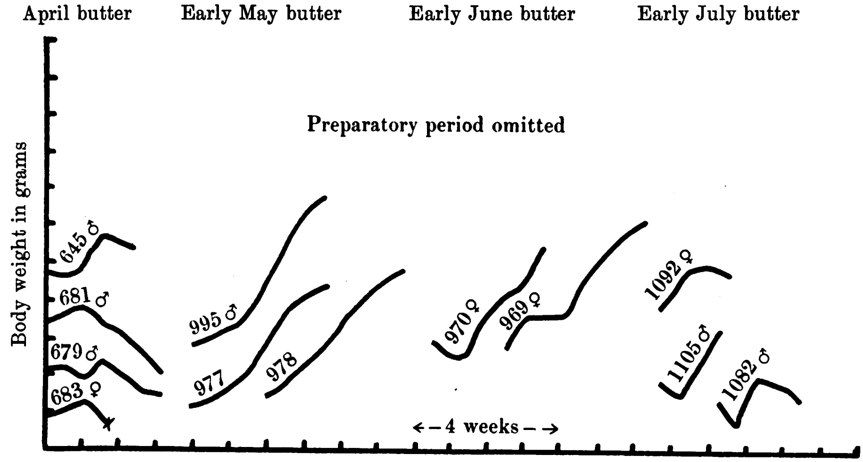

Fig. 3. Effect of diet on vitamin A value of butter. Curves 645, 681, 679, and 683 represent 0·2 gm. daily of butter from winter fed cows in April. The cows were put out to grass and the food value of the butter immediately rose as seen in the tests of 0·2 g. of the early May butter (curves 995, 977 and 978) and the early June butter (curves 970 and 969). The severe drought has caused a fall in the amount of vitamin present in July butter (curves 1092, 1105, 1082).

The farm in Buckinghamshire from which the samples of butter were obtained suffered very badly from the lack of rain so that before the beginning of July the pasturage was insufficient to support the cows, and it was necessary to fall back on cake. The effect of the drought in drying up the fresh pasture and of the cake feeding is already apparent in the marked falling off of the food value of the butter.

Further experimental confirmation of the relation of the food of the cow to the vitamin content of the milk has recently been brought forward in a preliminary communication by Hughes, Fitch and Cave [1921].

The storage and preservation of butter. We have investigated a number of samples of butter which have been in storage for various lengths of time, usually at low temperatures, and have not found them to possess a general lower average value than fresh samples. No sample which we investigated had, however, been stored for a longer period than eight months.

In these samples the value as a source of vitamin A appeared to be very much more dependent on the season at which it was placed in store, *i.e.* the diet of the animals at that season, than on the length of time it had been in storage. We have encountered one sample of Danish tinned butter which had been in tins for at least six months but which had a very high potency when tested for the vitamin. This sample had been derived from grass-fed cattle and was deeply tinted with the natural pigments derived from the green fodder. Our experience indicates that butter stored at low temperatures (below 10° C.) will not suffer any appreciable loss of vitamin if undue exposure of surface to air is prevented and the other conditions of storage are good[1].

We have also examined samples of beef fat (oleo-oil) from S. American grass fed cattle, and have found them to show as high a potency after many months' storage in tins as is possessed by good samples of butter fat.

If, on the other hand, the conditions of storage are such as to permit definite even if slow oxidation processes there will certainly be danger of loss of vitamin. This type of change is apparently very much more likely to occur in butters which have been made from "high acid" creams or which have been overworked [Hunziker]. The oxidation which occurs in such butters is according to Dyer [1916] that of the non-fatty constituents of the buttermilk, but we have encountered many cases where a marked loss of natural pigment due to oxidative bleaching of the lipochromes has occurred in such butters.

The loss of the natural pigments, carotene and xanthophyll, in butters on storage is in all the cases we have examined accompanied by a loss of vitamin, but this does not frequently occur unless the fats are exposed to light and warmth as well as air. We have tested one sample of the so-called "tallowy" butter produced by the prolonged exposure to air and light at room temperature, in which the oxidative processes had bleached all trace of pigment substances and given rise to an objectionable tallowy odour, and found that there had also been a complete destruction of vitamin A.

It is today well recognised that sweet cream butter stored at low temperature will not develop what is termed the "storage flavour" so readily as that prepared from pasteurised or neutralised cream. This development of "storage flavour" appears to represent a very early stage in the oxidation processes which lead on to the appearance of more objectionable odours, and eventually to rancidity [Rogers, 1913]. We have not noted any appreciable change in the vitamin value of butters showing the "storage flavour," but this may be due to the fact that our test method is not accurate enough to detect the slight change which might have occurred in the short time during which the changes had proceeded.

[1] This is in agreement with the results of Harden and Zilva (private communication) who found no detectable deterioration in the value of butter kept in cold storage for twelve months.

Rancidity. The groups of processes which together produce rancidity are very incompletely understood, but we know that the two most important types of reactions occurring, in most cases simultaneously, are, a hydrolysis of the glycerides and obscure changes of an oxidative character. When butter is so exposed that the second type of reaction is especially well favoured, we have found that rancidity is accompanied by a comparatively rapid loss of vitamin A. This is particularly marked if a large surface of the fat is exposed to air at moderate temperatures. On the other hand we have noted samples of butter protected from the light in which a certain degree of rancidity, as measured by the acid value, has developed without marked loss of vitamin. It would therefore appear that destruction of the accessory factor during rancidity will depend on the extent to which oxygen plays a part in the process.

In this connection we may mention the unpublished results obtained by Mr S. N. Ghose working in this laboratory, who has found that average samples of unadulterated Indian cow's ghee have approximately the same value as average samples of mixed butters. In the preparation of this product the freshly boiled milk is inoculated with sour milk and allowed to curdle. It is then churned and the fat is allowed to become rancid after which it is clarified. Whilst the rancidity develops the fat is kept out of contact with air and away from the light and heat.

Renovated butter. The practice of renovating butter which has become rancid and thereby reconverting it into an edible product appears to have died out in Europe. At any rate we were unable to ascertain where such products are generally made or to obtain more than one commercial sample. Apparently the manufacture of this product still exists to a considerable extent in America, most of our information having been derived from that source. According to Hunziker the usual American process involves prolonged aeration in large jacketed cylindrical tanks by means of a powerful current of air blown through a distributing rosette near the bottom until it has become "neutral," that is until the objectionable odours have disappeared together with most of the volatile fatty acids.

It appears to us highly improbable that any appreciable amount of vitamin present in the original butter could survive so drastic a process of purification. As we have remarked before, the employment of a soured milk to restore or develop the butter flavour would not add, in our opinion, any amount of vitamin A to the final product, unless where, as appears to happen in a few cases, whole milk is used.

Dr L. H. Lampitt has very kindly furnished us with information of the processes usually employed when butter was renovated in this country, and we have followed out the influence of one of these methods on the vitamin present in a sample of butter which had been fully tested in the fresh condition.

The sample of butter chosen was a good specimen of Danish dairy butter from cows out on green pasture, 0·2 g. per day of which supplied the requirements of the experimental animals for growth. A sample of this butter was allowed to become rancid by exposures to slight warmth and light for two weeks, after which it was again tested for activity. Its value was found to be very considerably lowered, 0·4 g. giving fairly satisfactory growth.

The rancid sample was then "renovated" by the following method. The fat was melted at low temperature and allowed to separate from the aqueous layer which was drawn off carefully. The liquid fat was then subjected to the passage of a current of live steam for half an hour to remove volatile components of an unpalatable nature. The deodorised fat was then shaken up vigorously with an equal volume of slightly soured separated milk, and the regenerated butter tested on rats in the usual manner. The results of the test show that little further loss of vitamin A had occurred as a consequence of the renovation.

A commercial sample of renovated butter fat which had not been reincorporated with milk was found to possess a high value as a source of vitamin A. No information of the method used in its preparation could be obtained. It possessed a marked tallowy odour and was most unpalatable, but the natural pigments of the butter had not been altogether destroyed.

SUMMARY.

1. The diet of the cow is undoubtedly the chief cause of variations in the amount of vitamin A in milk. Season, apart from the consequent diet, and breed are not the cause of any marked variation.

2. Colostrum normally proves to be richer in the vitamin than the later milk.

3. Butter appears to be less potent as a source of fat-soluble A than the same amount of fat supplied in the original milk. The loss as far as we can ascertain is partly mechanical and partly due to destruction.

4. Certain processes used in butter-making which involve exposure to air at high temperatures are believed to cause a loss of the A factor. This applies especially to some of the less frequently adopted methods such as "blowing" to remove objectionable odours.

5. Butter shows wide variations as a source of vitamin A. Most of this variation arises from differences in the diet of the cow. We have confirmed our previous observations on the low vitamin value of butter produced during the winter months from stall-fed cattle on dry feeds of hay, roots and cake. Even the drying up of pasturage in a hot summer may lower the value of the butter.

6. The storage of butter and its preservation by such methods as tinning do not appear to lower the vitamin A value unless changes of an oxidative character take place. Modern improvements in methods all tend to minimise loss from this cause.

7. Destruction of vitamin A occurs during the processes of rancidity if oxidation plays much part in this change, but considerable development of

free acid may occur without loss of the accessory factor if oxidation—as indicated by a bleaching of the natural pigment—is prevented.

8. The "renovation" of rancid butter will entail further loss of vitamin if the methods employed are likely to cause oxidation.

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REFERENCES.

Ayres and Johnson (1914). U.S. Dept. of Agric. Farmers Bulletin, 608.
Chick and Hume (1919). *J. Biol. Chem.* **39**, 203.
Coward and Drummond (1921). *Biochem. J.* **15**.
Daniells and Loughlin (1920). *J. Biol. Chem.* **44**, 381.
Drummond (1918). *Lancet*, Oct. 12.
Drummond and Coward (1920, 1). *Biochem. J.* **14**, 661.
—— (1920, 2). *Biochem. J.* **14**, 668.
—— (1920, 3). *Biochem. J.* **14**, 381.
Dyer (1916). *J. Agric. Res.* **6**, 927.
Hopkins (1912). *J. Physiol.* **44**, 425.
—— (1920, 1). *Biochem. J.* **14**, 721.
—— (1920, 2). *Biochem. J.* **14**, 725.
Hughes, Fitch and Cave (1921). *J. Biol. Chem.* **46**, 1.
Hunziker (1920). Butter Industry, Chicago.
McCollum, Simmonds and Pitz (1916). *J. Biol. Chem.* **27**, 33.
Medical Research Council (1919). Report 38.
Palmer and Eckles (1914). *J. Biol. Chem.* **17**, 191.
Rosenheim and Drummond (1920). *Lancet*, **1**, 862.
Rogers (1909). U.S. Dept. Agric. B.A.I. Circ. 146.
—— (1913). 3rd Internat. Congress Refrigeration (from Hunziker).
Zilva (1920). *Biochem. J.* **14**, 740.