XVIII. THE VITAMIN D ACTIVITY OF BUTTER.

III. AN ATTEMPT TO ELUCIDATE THE NATURE OF THE LABILE FACTOR IN BUTTER ANTIRACHITIC FOR THE RAT. THE ANTIRACHITIC POTENCY OF LARD, OLIVE OIL, EGG OIL AND THE FATTY ACIDS OF BUTTERS AND LARD.

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IN the previous paper of this series [Kon and Booth, 1934] we have summarised the evidence which points to the existence in butter of two factors antirachitic for the rat, the differentiation between the two factors being based upon the inability to recover one of them in the non-saponifiable residue of butter.

The present paper deals with a description of our efforts to elucidate the nature of the labile factor.

Our experiments were carried out in three main directions.

1. Firstly, we thought it advisable to investigate the effect of saponification on the antirachitic potency of eggs. With the exception of fish-liver oils and of some oils of marine animals, egg oil is probably the most concentrated natural source of vitamin D activity. Excluding such additions to the diet as cod-liver oil and fish meal it would seem that the exogenous sources of the antirachitic factor, or factors, available to the laying hen kept on open range are very similar to those placed at the disposal of the cow. Exposure to sunshine is common to both, as is also the consumption of grass, though in the latter case obviously in different relative quantities. We know of no marked difference in the qualitative or quantitative sense between the vitamin D activity, if any, of the other basal vegetable constituents of the diet of either the hen or the cow.

We therefore studied the effect of saponification on the antirachitic activity of eggs obtained from hens kept on open range and receiving no addition of either fish meal or cod-liver oil to their diet.

2. Secondly, we examined the fatty acid fraction of butters for antirachitic action.

Some ten years ago, Zucker and Barnett [1922-23], in trying to explain their failure to concentrate the vitamin D activity of butter in a manner successfully used by them in the preparation of an active concentrate from cod-liver oil, advanced the hypothesis that the antirachitic activity of butter as measured on the rat might be largely, if not solely, due to the action of the fatty acids of butter combining with the dietary calcium and causing the excretion of increased amounts of calcium from the gut in the form of calcium soaps. While we do not necessarily agree with Zucker and Barnett's explanation of the mechanism of fat action, we were nevertheless impressed, as they were, by the possibility of the saponifiable fraction of butter playing a rôle in the total antirachitic activity

of the latter. We have therefore planned and carried out experiments which, in our opinion, might help in elucidating the problem.

Zucker and Barnett's suggestion, as far as we understand it, was made in an attempt to reconcile the success of their method of concentrating the antirachitic factor of cod-liver oil with the failure of this method as applied to butter and other substances. It was not based on experimental evidence derived from the feeding to rats of the fatty acids of either butter or of the vegetable fats mentioned in their paper. We [Kon and Booth, 1934] have not only failed to extract by the above method the antirachitic activity of butter, but were equally unsuccessful in the case of cod-liver oil. On this basis, therefore, we see no *a priori* reason for ascribing the antirachitic activity of butter, partly or wholly, to its fatty acid moiety. Nevertheless, the peculiarities of the etiology of experimental rickets in rats make it imperative to investigate experimentally the possible rôle of the fatty acid fraction of butter. The rachitic condition in the rat cannot be brought about by a deficiency of vitamin D alone. A rachitogenic diet must also be grossly abnormal in the proportion of its mineral constituents in order to produce a good degree of rickets. It is well known [Karelitz and Shohl, 1927; Brown *et al.*, 1932; Shohl *et al.*, 1932; Key and Morgan, 1932] that the mere addition of phosphorus to a rachitogenic diet, or the withdrawal of a part of the calcium, produces the same effect as if an antirachitic substance had been added to the otherwise unchanged diet. It is, therefore, evident that any substance which would alter the calcium and phosphorus metabolism of the rachitic rat, either by increasing the excretion of calcium or by facilitating the uptake of phosphate from the gut, will behave, in bringing about a prevention or cure of the rachitic condition, in a manner indistinguishable from that of a true antirachitic agent.

The smaller the original potency of a butter the larger is the amount which it is necessary to feed to ascertain its antirachitic activity. It should be remembered that, as the fatty acids of butter weigh almost as much as butter itself, the amounts of fatty acids which it is necessary to administer to the rat when testing butters of low or moderate activity are quite considerable. There is ample evidence which has been lately summarised by Boyd *et al.* [1932] that fatty acids play a very definite rôle in the metabolism of calcium and phosphorus in the rat.

Under the second heading also must come our experiments in which we fed to rats the non-saponifiable residue simultaneously with the fatty acid fraction of butter. If that part of the activity of butter which is not recoverable after saponification is due to the antirachitic action of the fat, then such a "reconstituted" butter should regain its original activity.

We found it impossible to feed fatty acids separately from the diet by pipette as we feed butters. The higher melting-point formed one obstacle. A more serious one was the refusal of the rats to consume the fatty acids when given by pipette. These had therefore to be fed incorporated in the diet and as a check we thought it desirable to feed butter likewise incorporated in the diet.

3. Lastly, we fed to rats a vegetable fat, olive oil, and an animal fat, lard, in quantities comparable with those administered in the case of butters of low or moderate antirachitic activity.

Olive oil is generally recommended for use and is used as a diluent for cod-liver oil and a solvent for irradiated ergosterol. In small quantities high grade olive oil which has not been exposed to sunlight is antirachitically quite inert. The effects, if any, of large quantities of this fat would therefore, in our opinion, throw a valuable light on the fatty acid hypothesis of the antirachitic activity of butter.

The use of lard as a constituent of a rachitogenic diet composed mainly of purified foodstuffs has been suggested by Osborne and Mendel [1923] and Smith and Mendel [1930]. These authors advise the use of diets containing lactalbumin or edestin, starch, a phosphorus-free salt mixture and from 0 to 24 % lard. For this reason we chose lard as a representative animal fat which might be considered to be antirachitically inert.

EXPERIMENTAL.

Methods.

1. *Egg oil.* Fresh eggs were obtained at the end of June 1933 from Light Sussex hens on open range and receiving a grain mixture containing meat meal but no cod-liver oil or fish meal. Eggs were also collected at the end of October 1933 from the same hens receiving the same diet.

The yolks of the eggs were carefully separated from the whites, and after adding an equal volume of 8 % NaCl [Jukes and Kay, 1932] they were extracted three times with ether, the third extract being almost colourless. The extract was washed twice with water, dried over anhydrous Na_2SO_4 and the ether evaporated after filtering. For feeding, the egg oil was diluted with olive oil in the usual way. After saponification of 10 g. of egg oil the mixture of soaps was extracted seven times with ether using one portion of 75 ml. and six portions of 50 ml. This number of extractions was necessary in order to remove the bulk of the colouring matter (xanthophyll). It is very probable that a smaller number of extractions would have effected a quantitative recovery of the antirachitic factor. The non-saponifiable residue was dissolved in olive oil for feeding as previously described.

2. *Fatty acids.* All the fatty acids discussed below were prepared by the following method. After removal of the non-saponifiable residue from saponified butter or lard the soaps were acidified with sulphuric acid and the fatty acids extracted three times with ether. The ethereal solution was washed twice with water and dried over anhydrous Na_2SO_4 . The ether was then removed, firstly on the water-bath and then under diminished pressure. This method of recovery is not quite quantitative, losses occurring in the lower volatile and water-soluble fatty acids. For feeding, the fatty acids were incorporated in diet 2965 of Steenbock by dissolving them in ether, mixing the solution with the diet and subsequently allowing the solvent to evaporate. The fatty acids derived from the following butters and lard were prepared and fed¹: summer butter; irradiated butter; autumn butter; high-level yeast butter; winter butter; home-rendered lard. Records of food intake of the several groups of rats receiving these fatty acids were kept. As in the case of all other substances tested, each fatty acid and level of fatty acid was fed to groups of 4 rats kept in one cage. The food records give therefore the average amount of fatty acid consumed by any rat in the group. It will be remembered [Kon and Booth, 1933; 1934] that all substances fed separately from the diet were administered on weekdays only, while the substances incorporated in the diet were given seven times a week. Therefore to give a true impression of the antirachitic activity, if any, of the fatty acids we give in the following table average weekly consumptions. The fatty acids were usually fed in the same experiments as those involving the feeding of the corresponding original fats and their non-saponifiable residues. Whenever several samples of butter were saponified in the course of one feeding test, fresh

¹ The corresponding butters were discussed in our previous paper [Kon and Booth, 1934].

samples of the corresponding fatty acid-containing diets were prepared on the same days as those on which the changes were made in the feeding of the fats and non-saponifiable residues.

When fatty acids were fed to rats simultaneously with the administration of the non-saponifiable residues, the fatty acids were fed exactly as described above, and the non-saponifiable residues were administered six times a week in the usual way.

For feeding in the diet the butter-fat was melted and poured over the requisite amount of the 2965 rachitogenic diet and was then well kneaded in by hand until a perfectly homogeneous material was obtained. The bulk of this was stored in a cold place. These diets were fed and food intake was recorded in the same way as in the case of fatty acids.

3. (a) *Olive oil*. The olive oil was fed by pipette separately from the diet in an exactly similar manner to the butters. It was a high grade first pressing Italian oil which had been used by us with complete satisfaction as a diluent for cod-liver oil and the International Standard of Vitamin D, and as a solvent for the various non-saponifiable residues and other substances requiring dilution.

(b) *Lard*. Three samples of lard were tested, two of them were purchased from a local grocer and were both of American origin. It is quite possible that they may have been both from the same consignment, but we prefer to consider these lards as separate specimens. The second sample was from a bulk labelled: "White Rose Pure Lard, Dold Quality, Buffalo, U.S.A."

The third sample was a lard rendered by us from the leaf fat of 4 pigs slaughtered at bacon weight at the end of September 1933 when 7 months old. For the last 4 months of life they had been kept indoors without access to sunlight and grass and had been maintained on the following diet:

Barley meal	48.0 %	Middlings (Weatings)	35.0 %
Soya-bean meal	6.0	Meat meal	4.0
Dried whole milk	5.0	Ground limestone	1.75
	Sodium chloride	0.25 %	

It is reasonable to suppose that the vitamin D reserves, if any, of these animals were of a low order. For rendering, the chopped fat was heated in a pail immersed in boiling water and the fat filtered through cheese-cloth, allowed to settle and set, remelted and filtered through a fluted filter.

The first sample of lard was fed only as such. The second and third samples were fed both before and after saponification. The non-saponifiable residues were extracted four times with ether. The lards were given by pipette separately from the diet. One level of the first sample was fed mixed in the diet.

It will be seen from Table I that there is no difference between the anti-rachitic effects of doses of summer egg oil and those of corresponding levels of the non-saponifiable residues of the same oils. On the other hand the highest of the three levels tested did not afford more than a slight degree of protection. A reference to the previous paper of this series [Kon and Booth, 1934] will show that, although in the case of summer butter and of cod-liver oil butter there was no difference between the antirachitic effects of the butter and of its non-saponifiable residue at the lowest level of intake, which gave an ash percentage similar to that produced by the highest level of egg oil, definite differences could be noted at the higher levels. In the light of our experience with summer egg oil the levels of autumn egg oil were better selected, but unfortunately an error was made in the dilution of the non-saponifiable residue which was made up to twice

Table I. *Summer and autumn 1933. Prophylactic effect of various egg oils, lards, butters and their non-saponifiable residues; olive oil; fatty acids of lard and butters; fatty acids and non-saponifiable residue of butter fed together; butter incorporated in the diet. Percentage of ash in dry defatted femora and humeri.*

The figures for ash percentages are averages of duplicate determinations on groups of 4 rats.

The dosages of non-saponifiable residues are expressed in equivalents of the butter or lard from which they were derived.

Substance tested	Dosage g.	Ash %	Substance tested	% in diet	Av. weekly intake of sub- stance per rat	Ash %
Summer egg oil	0-001	30-5	Summer butter	Fed separately	3-0	49-5
Summer egg oil	0-003	34-1	Summer butter fatty acids	6-25	3-0	37-6
Summer egg oil	0-006	38-9	Negative controls at this time	—	—	32-0
Non-saponifiable residue of summer egg oil, equiv. ...	0-001	30-1	Irradiated butter	Fed separately	0-06	46-9
Non-saponifiable residue of summer egg oil, equiv. ...	0-003	34-0	Irradiated butter fatty acids	0-20	0-15	30-9
Non-saponifiable residue of summer egg oil, equiv. ...	0-006	38-2	Winter butter	Fed separately	4-20	47-3
Autumn egg oil	0-0115	41-2	Winter butter fatty acids	7-00	3-23	35-8
Autumn egg oil	0-0345	51-5	Negative controls for irradiated butter and winter butter	—	—	34-9
Non-saponifiable residue of autumn egg oil, equiv. ...	0-0230	42-2	Autumn butter	Fed separately	3-00	47-1
Non-saponifiable residue of autumn egg oil, equiv. ...	0-0690	52-0	Non-saponifiable residue of autumn butter	Fed separately, equiv.	3-00	39-1
Olive oil	1-0	37-4	Autumn butter fatty acids	6-25	3-26	36-7
Negative controls for olive oil	—	30-1	Autumn butter fatty acids + non-saponifiable residue	6-25	3-37	41-2
American lard, 1st sample	0-25	43-9	Autumn butter in diet	6-25	3-78	43-3
American lard, 1st sample	0-50	51-2	Autumn butter	Fed separately	6-00	52-9
American lard, 1st sample	1-00	51-5	Non-saponifiable residue of autumn butter	Fed separately, equiv.	6-00	40-3
American lard, 1st sample incorporated in the diet, approximately	1-50	49-2	Autumn butter fatty acids	12-50	6-16	40-3
American lard, 2nd sample	0-5	48-5	Autumn butter fatty acids + N.S.R.	12-50	5-75	50-2
American lard, 2nd sample	1-0	51-3	Autumn butter in diet	12-50	6-80	48-5
Non-saponifiable residue of American lard, 2nd sample, equiv. ...	0-5	41-5	Negative controls for autumn butter	—	—	33-2
Non-saponifiable residue of American lard, 2nd sample, equiv. ...	1-0	45-4	High-level yeast butter	Fed separately	0-06	46-0
Home-rendered lard	1-0	48-0	High-level yeast butter fatty acids	1-25	0-75	43-1
Non-saponifiable residue of home rendered lard, equiv. ...	1-0	34-5	Negative controls for high level yeast butter	—	—	32-0
Negative controls for American lard, 2nd sample and for home-rendered lard	—	34-9	Home-rendered lard fatty acids	12-50	5-60	39-7
			Negative controls for these fatty acids	—	—	33-2

the concentration of the original oil. The results might be interpreted as indicating a certain loss of the antirachitic potency during the course of saponification. We would prefer for the time, however, to consider these figures as inconclusive. It should be mentioned here that Hess and Weinstock [1924] found that the non-saponifiable residue of egg-yolk was antirachitically active and obtained a preparation about 90 times as concentrated as the original yolk. We are planning to investigate the effect of saponification on eggs from the same source as those obtained and tested by us in summer and autumn, but collected in February when their antirachitic potency should be at its lowest.

The feeding of the olive oil at a 1 g. level had a definite effect upon the severity of the rachitic condition. It will be noticed that the bones of rats receiving this quantity of olive oil contained 7 % more ash than those of the negative controls.

The results of feeding the first sample of the American lard were quite startling. The antirachitic effect of this lard was, if anything, better than that of the summer butter fed at the same time. (The figures for this butter are given in the previous paper [Kon and Booth, 1934].) No records of food consumption were kept in the case of lard incorporated in the diet, but an average consumption of 8 g. per day would imply the ingestion of 1.5 g. of lard. It would appear that lard incorporated in the diet is less antirachitically effective than when administered separately from it.

The second sample of American lard was also, comparatively speaking, very potent and gave results similar to those produced by the first sample. The non-saponifiable residue gave definitely lower values than the lard itself, and the degree of loss was very similar to that which occurred in the case of summer butter.

The home-rendered lard was less potent than the two American lards. Very marked loss took place here after saponification, the rats receiving the non-saponifiable residue being indistinguishable from negative controls.

Drummond *et al.* [1920], in a paper written before vitamins A and D were differentiated, stated that the addition of lard obtained from pigs receiving an ample supply of the fat-soluble vitamin definitely increased the growth and improved the condition of rats kept on a diet deficient in this factor, while lard from a pig without such supply of vitamin failed to do so. Quite recently (since this work was started) Read and Bailey [1933] have stated that some lards may contain an appreciable amount of vitamin D, while others contain an inappreciable amount of this factor.

On reviewing the antirachitic effect of fatty acids it is apparent that their action, though slight, is unmistakable. With the sole exception of those of high-level yeast butter the fatty acids, even at a level of 1 g. daily, do not increase the ash beyond 40 %. The case of the high-level yeast butter fatty acids requires some explanation. These fatty acids fed at a level of approximately 0.12 g. daily produced an ash percentage of 43.1, quite definitely higher than that given at the same time by the negative controls. On the other hand the degree of protection afforded by this amount of fatty acid is lower than that given by one-twelfth this amount of the original butter. This was the only fatty acid of this series which was separated from the non-saponifiable residue by only three ether extractions. If only 5 % of the non-saponifiable residue were left in the fatty acids, which under these circumstances is quite possible, the activity of this sample of fatty acids would be accounted for.

In the case of the remaining fatty acids which had all been separated from their non-saponifiable residues by four or, in the case of autumn butter, five extrac-

tions, no activity ascribable to the non-saponifiable fraction would seem to be left in the acids. The activity of the acids does not seem to bear any relation to the original total activity of the fat but appears to be proportional to the amount of fatty acid consumed. Thus for example the fatty acids of irradiated butter, though fed at almost three times the level of the original irradiated butter, gave an ash percentage actually lower than that given by the negative controls for this group of experiments, though the irradiated butter fed at the lower level afforded almost complete protection.

The combination of fatty acids and non-saponifiable residue of autumn butter gave, at the higher level of 1 g., only a slightly lower value than that produced by 1 g. of untreated butter. On the other hand the combination of fatty acid and non-saponifiable residue proved definitely less effective at the lower level of 0.5 g. than the corresponding amount of untreated butter.

When the butter was incorporated in the diet instead of being fed separately from it, the ash percentages were definitely lower in the former than in the latter case, although on the basis of food consumption slightly more butter was consumed by the animals which received it as an admixture to the diet. As already stated we noticed this phenomenon also in the case of lard.

DISCUSSION.

In order to appraise the significance of the experimental results described in this paper the following points should, in our opinion, be taken into consideration.

Firstly, with regard to its antirachitic properties lard behaves in a way very similar to that of butter. Different samples of lard display activities ranging from that of winter to that of summer butter, the antirachitic potency being lost on saponification in a way closely resembling that found in the case of butter, the loss being less marked in the more potent sample.

Secondly, when fed at 0.5 and 1.0 g. levels all fatty acids seem to have had a definitely measurable antirachitic effect. It seems extremely doubtful whether this effect can be attributed to incomplete removal of the stable vitamin D, as the amount of total activity which would have to remain in the fatty acids in order to produce such an effect would be far too great in the case of butters of originally low activity. Even on the unlikely assumption that, after 4 or 5 extractions with ether, 5 % of the orthodox activity had been left in the fatty acids, the amount so left in the case of autumn butter or of home-rendered lard would be quite insufficient to raise the ash percentage so definitely above the level of the negative controls. The completely negative results in the case of the fatty acids of irradiated butter, which were fed at a level relatively (on the basis of original potency) more than double that of the fatty acids of the butters and lard of low activity, also point in the same direction.

Thirdly, when butter is incorporated in the diet, its antirachitic effect is definitely lower than that of an equivalent amount of butter fed separately. The same phenomenon can also be noticed in the case of lard. It should be emphasised here that this difference in potency according to the method of feeding cannot in itself explain or account for the very marked drop in potency caused by saponification. A comparison of the ash percentages produced by 0.5 and 1.0 g. of autumn butter fed in the diet with those for corresponding levels of the same butter after saponification given in Table I bears out this statement. As already mentioned we have been technically unable to feed the fatty acids separately from the diet. Should they behave in a way similar to that of the original butter, then their antirachitic effect would be more marked when fed separately.

Fourthly, a combination of the fatty acids and non-saponifiable residue of autumn butter elicits an antirachitic effect superior to that produced by either component fed separately. As the fatty acids were fed in the diet we think that the antirachitic effect of such a "reconstituted" butter should be compared with that of corresponding amounts of the original butter also incorporated in the diet. When this is done it will be found that the difference in antirachitic effect is so slight as to be well within the limits of experimental error. In other words, it would seem that the total activity of butter (of this particular sample of autumn butter in any case) can be recovered quantitatively after saponification when the fatty acid moiety of the butter is recombined with the non-saponifiable fraction.

We have proved to our entire satisfaction in the two previous papers of this series [Kon and Booth, 1933; 1934] that the loss of potency following saponification evident in certain butters cannot be attributed to incomplete extraction of the non-saponifiable residue. Bearing this in mind and adding the evidence just discussed we can arrive at the following alternative suggestions as to the nature of the labile antirachitic factor of butter.

A. The first possibility would be to accept the existence in butter and in lard of a novel antirachitic factor unstable to saponification, and in general more labile than the usual vitamin D. This factor would appear to lose a part of its potency immediately after the butter or lard is mixed with the 2965 rachitogenic diet even when it is kept thereafter at a fairly low temperature; conditions under which irradiated ergosterol and cod-liver oil are stable for longer periods. The existence of such a factor seems quite conceivable were it not for the fact that it is difficult to account on this basis for the slight but unmistakable residual activity of the fatty acids. Also, if the existence of the novel factor should be the true explanation, it is very difficult to understand why a combination of fatty acids and non-saponifiable residue should have virtually the same antirachitic activity as corresponding levels of butter incorporated in the rachitogenic diet.

B. It might also be suggested that the antirachitic activity of butter was the resultant of two factors, namely, the activity of vitamin D *sensu stricto*, whatever this term implies, and of the antirachitic action of fat. In other words it might be suggested that the rat gives, because of its physiological peculiarities, an enlarged and distorted picture of the "true" antirachitic activity of butter, reflecting not only a real antirachitic factor pharmacologically active in different species, but also a non-specific stimulus antirachitic only in the narrow "rat" sense of the word. There is much in our experimental evidence in favour of such a view. The residual activity of the fatty acids, remarkably constant in the case of butters and lard and independent of the activity of the parent substance, and the possibility of reconstructing the original activity of butter by combining fatty acids and non-saponifiable residue speak strongly in favour of such an hypothesis. This explanation has also in its favour the argument of simplicity, as it avoids the necessity of adding another general antirachitic factor to the two already existing. It explains without difficulty why lard should behave in a similar way to butter and also accounts for the slight activities of various vegetable oils reported in the literature and found by us in the case of olive oil.

If also that part of the antirachitic activity of butter which cannot be recovered after saponification is not due to the presence of a specific antirachitic factor but to the action of fat, it is obvious that any attempt to increase the vitamin D activity of butter [Kon and Booth, 1934] would result only in an

increase in the orthodox vitamin D and not in the novel factor. This, in our experience [Kon and Booth, 1934] is actually the case.

Our finding that either butter or lard incorporated in the diet is less active than corresponding levels fed separately is not incompatible with the suggestion that a part of the antirachitic activity of these substances is due to their fatty acids. The antirachitic action of fat in the case of the rat must be due to the influence of the fat on the calcium and phosphorus metabolism, and it seems quite permissible to assume that the effect of one massive dose daily will be different from that of the same amount distributed over a period of 24 hours.

We have already mentioned that Zucker and Barnett [1922-23] suggest as an explanation of the mechanism of the action of fat the formation of insoluble calcium soaps. Recent work [Boyd *et al.*, 1932] would rather favour the view that the beneficial effect of fat is due to the creation in the gut of a p_H favourable to the uptake of minerals. We do not want to theorise on this point but wish to postpone judgment pending the collection by us of experimental data on the effect of various fatty acids on the calcium and phosphorus metabolism of the rachitic rat.

It would seem to us, that on the whole the arguments in favour of fat playing a rôle in the antirachitic activity of butter are quite serious enough to deserve careful consideration.

C. There is just a possibility that both fat and a novel factor are responsible for the antirachitic activity of the labile factor of butter and lard in the case of the rat. Insufficient experimental evidence prevents us at present from discussing this point any further.

We are led to believe that, unless the labile factor of butter is definitely proved to be antirachitic also for other species besides the rat, the only safe course for ascertaining the vitamin D activity of butter is to submit it to saponification and feed to rats the non-saponifiable residue only. Even then in order to decide *a priori* what might be the antirachitic value of such butter in human nutrition, it would be necessary to find out whether the orthodox antirachitic factor present therein is identical with irradiated ergosterol or with the vitamin D of cod-liver oil, or whether it is altogether different from these two.

We [Kon and Booth, 1933; 1934] have examined various butters by the widely used prophylactic method, making use of what is practically the official diet (the 2965 diet of Steenbock) and administering graded doses of butter separately from the diet, a method used to our knowledge by the majority of present day workers. We have very good reason to believe that, in the case of many butters, especially those collected in autumn and winter, values so found by this generally used technique for their alleged vitamin D content may be anything up to 4 or 5 times too high.

It may be also remarked that similar results were obtained by the line test technique as far as this is possible with butters of low potency [Kon and Booth, 1933; 1934].

We have purposely refrained from any discussion of the clinical antirachitic value of butter, but we think that our results may perhaps suggest a plausible explanation of the discrepancy of opinion existing between the clinician and the student of nutrition with regard to the antirachitic value of butter.

SUMMARY.

1. When tested on rats, lard was found to possess an antirachitic activity of the same order as that of butter, a sample of home rendered lard being almost equal in that respect to winter and autumn butter, while a sample of American

lard was found to be fully as active as summer butter. As in the case of butter, the activity of lard is impaired by saponification, the degree of loss being greater in the case of the lard of lower activity.

2. Fatty acids prepared from various butters were found to exhibit a slight but definite antirachitic activity which could not be attributed to incomplete removal of the non-saponifiable residue. This activity was roughly proportional to the amount of fatty acid fed and was independent of the original activity of the butter.

3. When butter was incorporated in the diet, it was found to produce a lower antirachitic effect than that given by a similar amount of butter fed separately from the diet.

4. In the case of an autumn butter, which after saponification had lost a large part of its original antirachitic potency, this potency could be recovered practically completely when the administration of the non-saponifiable residue was accompanied by the feeding of the fatty acid incorporated in the diet.

5. Although the loss of potency observed by us in various samples of butter could be tentatively explained by assuming the existence of a new labile antirachitic factor, we are inclined to believe at present that this phenomenon could be at least as adequately, and certainly more simply, explained by the antirachitic effect on the rat of fat as such.

6. If this explanation is, as we believe, correct, then, unless the labile factor of butter is definitely proved to be antirachitic also for other species besides the rat, values obtained by accepted methods for the antirachitic activities of butters are about 4 to 5 times too high in winter and autumn and about twice too high in summer. On the other hand values obtained by direct feeding of highly potent butters giving complete protection at a daily level of 10–30 mg., such as irradiated butter or butter yielded by cows receiving large amounts of irradiated yeast, are correct.

7. On the same basis it is suggested that while the general antirachitic value of the labile factor is still in question the only safe course in ascertaining the vitamin D activity of butter is to submit it to saponification and feed to rats the non-saponifiable residue only.

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