LXIX. THE EFFECTS OF HEAT AND AERATION UPON THE FAT-SOLUBLE VITAMINE.

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A KNOWLEDGE of the conditions which affect the stability of vitamines is not only of immediate practical importance in connexion with the commercial and domestic treatment of foods, but is, clearly, not without value in setting certain limitations to hypotheses which may be framed as to the nature of these substances, and also in giving guidance when attempts are made to isolate them.

There is at the moment less certain knowledge of this kind in the case of the "fat-soluble A" than in those of the two other recognised vitamines. Thus Steenbock, Boutwell and Kent [1918] came to the conclusion that the substance is readily destroyed by heat, and, later, Drummond [1919] from the results of his earlier experiments came to the same conclusion. Osborne and Mendel [1920], on the other hand, have recently confirmed earlier results of their own which indicated that it is resistant to heat. The investigation to be described in the present paper—to the results of which public reference has already been made [Hopkins, 1920]—confirms Osborne and Mendel's work by showing that the vitamine is relatively resistant to heat. It has demonstrated, on the other hand, that this nutritive factor is rapidly destroyed by exposure to atmospheric oxygen at temperatures ranging from 15° to 120°.

EXPERIMENTAL.

While the main purpose of this paper is to describe experiments which bear upon the relative importance of temperature and aeration as factors concerned in the destruction of the vitamine A in butter, it will be useful to refer first very briefly to an investigation undertaken with a somewhat different aim.

I. *Is there any Destruction of Vitamine during the Commercial Purification of Vegetable Fats?*

When vegetable fats are prepared for use in foods such as margarine they are of course first purified. It is a point not without importance to decide whether the processes applied have or have not destroyed vitamine which may have been present, to some extent at least, in the original crude fats.

Some two years ago I was asked by the firms mentioned at the end of this paper to investigate this question. The fats employed were palm kernel oil and ground nut oil, each respectively in the crude condition and purified for use. A large number of rats was used in testing the point, but it is not proposed to describe the results in detail. The practical issue was that in the case of neither fat could any difference in vitamine content be found between the crude and purified material. The average growth curves of the animals fed respectively upon the crude and pure fat were almost super-imposable, and the average curves of those upon the palm kernel oil were exactly similar to those upon ground nut oil. The results indeed may be grouped together as a whole to yield statistics regarding the behaviour of the rat-stocks in my laboratory when on a diet free from—or greatly deficient in—the fat-soluble vitamine. Except for the fats the diet was identical with that employed in all the experiments mentioned in this paper. In the case of 60 of the animals used, coming from the same or strictly comparable stocks, the average date upon which growth ceased was the 25th day, and in respect of this datum individual animals were closely grouped round the mean. The date of death varied of course much more widely. On the average it was the 56th day. 7 % were dead on or about the 30th day; 12 % died between the 40th and the 50th, 54 % between the 50th and 60th, and 27 % outlived the 60th day. These data, since they were obtained from rat-stocks which were essentially the same as those used for the experiments described in the next section, are of value for comparison.

II. *The Effect of Heat and Aeration on the Vitamine Content of Butter.*

In all the experiments carried out in this connexion the diet comprised highly purified caseinogen, potato starch, cane sugar, butter. The mineral supply was Osborne and Mendel's salt mixture with the addition of 0·10 % potassium iodide and a few milligrams per cent. of sodium fluoride. The butter was always first filtered and, however subsequently treated, formed always 15 % of the food.

The number of animals employed in the whole investigation was large. The growth curves if given in every case would occupy much space, so that certain of the observations must be dismissed with a verbal description. Most of the experiments were made comparative; balanced sets of animals being fed side by side and the one significant factor alone varied in their respective diets. Many authors seem content to compare growth obtained upon a given experimental dietary with the accepted "curve of normal growth" and not with a curve obtained at the time of the experiment from comparable animals on a normal dietary. It is the experience of my laboratory that the curve of normal growth varies both with the season and with the stock. Osborne and Mendel have, I think, commented upon the influence of season on their experimental results. With my stock we seem always to obtain the clearest and most satisfactory results in respect of growth curves during the periods from the beginning of April to the end of September. This is not a matter of temperature as the laboratory is always uniformly warmed. In the present investigation the comparison has frequently been, not between animals upon the experimental dietary and others upon normal food, but between one set upon fat treated in a given fashion, and another set upon the fat treated differently. Such sets have always been fed side by side.

There is doubtless even greater rigor of proof in the technique used by some others which consists in attempting to restore animals which are presumed to have failed owing to the absence of this or that constituent from their diet by a subsequent supply of that constituent. This method we have occasionally used in the present investigation as in Exp. 6. There are some advantages in the comparative method however.

Experiments 1 *and* 2. Early in 1919 eight rats averaging 57 g. in weight were placed upon a dietary in which the fat was butter previously heated in a steam autoclave for four hours at 120°. Eight similar rats had the same food but with the butter heated at 120° in an oil bath and simultaneously aerated for 12 hours with a brisk stream of air. The fat entirely lost its yellow colour. In the former set, three of the animals exhibited somewhat slow growth from the first, and died before the 60th day, but the remaining five grew vigorously and remained in good health for 160 days when the experiment was stopped. In the case of the second set all the animals failed to grow after the 21st day, five deaths had occurred by the 50th day, while the survivors afterwards developed keratomalacia and were killed. At about the same time eight other rats received butter heated for 12 hours to 80°, in such a way that a considerable surface of the fat was exposed to air. Growth was slow from the first; four deaths occurred about the 60th day, and a fifth occurred shortly afterwards. The three survivors lived to the 140th day when the experiment was stopped; but they had long ceased to grow, and were in poor condition.

These preliminary experiments were sufficient to offer a strong suggestion that aeration is an important factor in the destruction of the vitamine, but the comparison was faulty in the case of the first two sets of animals, since the aerated fat was heated thrice as long as the unaerated.

Experiment 3. In this case the comparison was rigorous, but the experiment was begun when but few young rats happened to be available for the purpose, so there were only four animals in each of the sets compared. The butter was in the one case autoclaved at 120° for four hours, and in the other heated to the same temperature and period but at the same time thoroughly aerated. The first set grew vigorously and remained in perfect health; the set on the aerated fat ceased to grow about the 25th day, and all died about the 50th day.

Experiment 4. This was a crucial experiment. Two carefully balanced sets of rats were taken, with ten animals in each set. One set received the standard diet with butter previously heated to 120° in the autoclave for four hours; the other set had the same diet with the butter heated at the same temperature

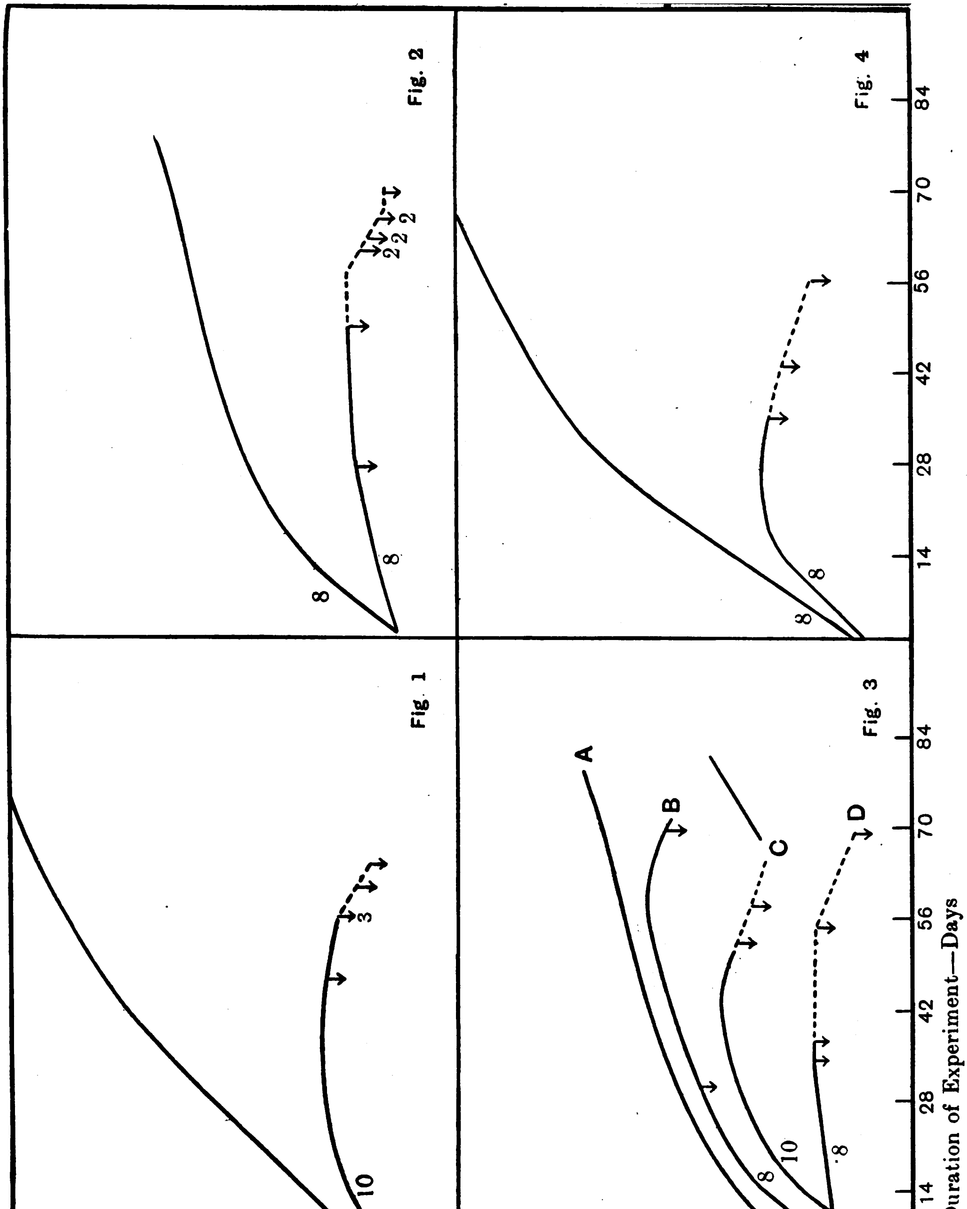

Fig. 1. (Experiment 4.) Upper curve shows average growth of 10 rats on a dietary containing butter heated out of contact with air for 4 hours at 120°. Lower curve shows growth of 10 comparable animals depending upon butter heated for the same time and at the same temperature but simultaneously aerated.

Fig. 2. (Experiment 5.) Curves as in Fig. 1, but the animals were fed with butter respectively autoclaved and aerated for 12 hours at 120°.

Fig. 3. (Experiments 6, 7.) Curve A, animals fed with butter aerated for 1 hour at 120°; B, aeration 2 hours; C, aeration 4 hours; D, aeration 16 hours. The unbroken line continuing Curve C shows the growth of surviving rats when put upon fresh butter.

Fig. 4. (Experiment 8.) Lower curve: animals depending on butter exposed in thin layers to air at ordinary temperatures. Upper curve: animals on butter preserved out of contact with air.

The arrows indicate deaths. When a death appreciably affected the average weight of the group the continuity of the curve is maintained by calculating the weight changes of the survivors on a percentage basis. Change in the curve from a continuous to a dotted line indicates that this has been done. The figures adjacent to the curves indicate the number of animals comprised in each set; those below the arrow give the number which died on a particular date.

and for the same time but with simultaneous aeration. The aeration as in other experiments was carried out in a flask immersed in an oil-bath. By means of a filter pump a stream of air was drawn through the melted fat at the rate of four or five bubbles per second.

The average growth curves obtained in this experiment are shown in Fig. 1, and in Fig. 5 are shown the curves of individual growth. In the latter the curve of each animal in one set is reproduced side by side with that of its representative in the other set, each being of the same sex and so far as possible of the same initial weight. (Two extra rats were in the set receiving aerated fat, for which, when the experiment was started, no exact duplicates could be found. These appear in the curves of individual growth, but they did not contribute to the average curve.)

None of the animals upon the autoclaved butter displayed keratomalacia during the course of the experiment. Of those receiving the aerated butter, three showed symptoms of this disorder on the 40th day, three on the 42nd, three on the 53rd, and one on the 64th day.

Experiment 5. The conditions in this experiment were exactly those of No. 4, but the butter was respectively autoclaved and aerated for 12 hours instead of 4 (120°). Curves of average growth in Fig. 2. To save expense of reproduction individual growth curves are omitted in the case of this and subsequent experiments. The degree of variation seen in Exp. 4 (Fig. 5) is quite typical of other experiments. There was no keratomalacia in the case of the animals receiving the fat heated out of contact with air. Of those on the aerated butter all developed keratomalacia between the 40th and 43rd day.

Experiment 6. Rats belonging to the same stock were divided into three groups with eight animals in each, the groups being balanced in respect of sex and weight. The diet was the same as in all other experiments, but with the butter aerated at 120° for one, two and four hours respectively. A fourth set of animals comparable with the others in respect of sex and weight but from a different stock, and fed at an earlier period, took the diet with butter aerated at the above temperature for 16 hours. The resultant growth curves are shown in Fig. 3.

There was no keratomalacia among the animals receiving fat aerated for one or for two hours; but, in marked contrast with the animals of Exp. 4, there was also none when the butter had received four hours' aeration. The rats concerned came from a stock quite distinct from that employed in Exps. 4 and 5. In the case of the group upon the fat aerated for 16 hours eye trouble developed in several of the animals, but no accurate record of its incidence was kept in this case.

Experiment 7. Filtered butter was kept at 80° and aerated with a brisk stream of air for 12 hours. Eight rats received the usual dietary with this aerated butter as its fat constituent.

The average growth curve corresponded so exactly with that of the animals

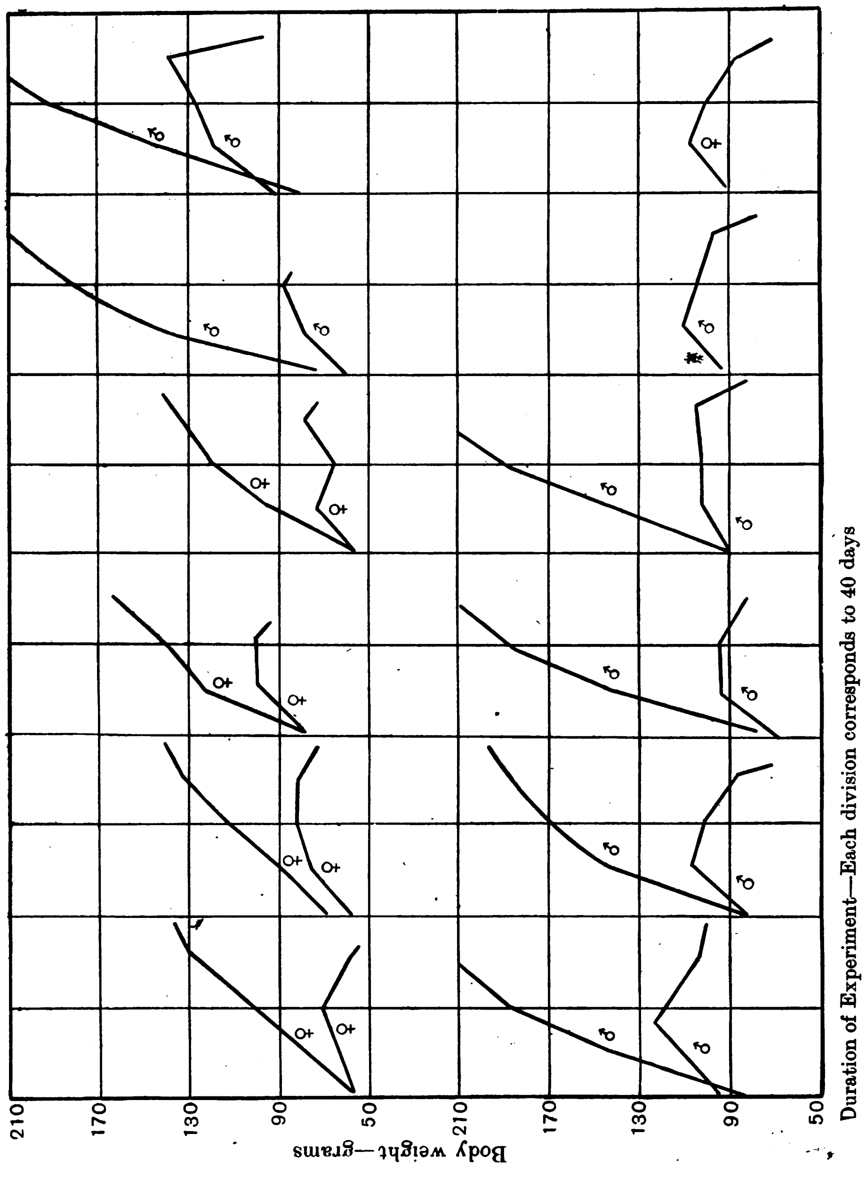

Fig. 5. Growth diagrams of individual rats in Experiment 4.

The upper line in each case shows the changes in body weight of an animal from the group receiving butter heated but not aerated. The lower line refers to an animal corresponding to the first in sex and initial weight, but from the group which depended upon aerated butter. Two rats in the second group were not represented in the first.

In every case the end of the lower curve marks the death of the rat.

which in Exp. 6 received butter aerated at 120° for four hours (Fig. 3, Curve C) that it need not be reproduced. Keratomalacia occurred in four cases between the 40th and 60th days.

Experiment 8. Filtered butter was exposed in thin layers at ordinary room temperatures, varying between 15° and 25° (during May, June and July) for periods of about a week. It had by this time lost all, or nearly all, its pigment. The fat so treated was added to the standard diet in the same proportion as in all other experiments, and the animals receiving it were compared with another set upon butter kept *en masse* for corresponding periods without exposure to air or light. The average growth curves (from eight rats in each set) are shown in Fig. 4. The animals were of the stock used for Exp. 6, different therefore from those of Exps. 4 and 5. It is noteworthy that though growth and nutrition failed on the exposed fat keratomalacia developed in one rat only and not before the 65th day.

Discussion of Results.

It will be seen that the experiments described show in the clearest way that while the fat-soluble A factor is relatively resistant to the effects of heat alone, it is easily destroyed by aeration, presumably because it is a substance prone to oxidation.

It was on these points, and on these alone, that information was sought when the experiments were first planned, and for such a purpose the comparative method used seemed to be the best. The animals, in balanced sets, were fed side by side, aeration or non-aeration of their fat supply being the only difference in the treatment of the animals in the sets so compared. The difference in their behaviour was always unequivocal and most striking.

My experiments, however, give no quantitative information as to the *rate* of the destruction of the vitamine, whether by heat alone or with combined aeration at a given temperature. For this extended experiments and a different technique are called for.

The observations indicate that four hours' exposure to 120° does not, in the absence of air, sufficiently reduce the vitamine content of butter to make the heated product any less efficient than normal butter for maintaining rats, at any rate when the fat forms 15 % of their food (Exp. 4). Twelve hours' exposure to the same temperature seems undoubtedly to involve some destruction, but in the absence of air the vitamine is far from being completely destroyed (Exp. 5). On the other hand, aeration for four hours at 120° destroys the greater part of the vitamine, and exposure for 12 hours to the same condition involves what would seem to be almost complete destruction (Exps. 4, 5, 6). At the same temperature, one hour's aeration renders butter definitely less efficient for maintenance than unheated butter, and an increase in the destruction after two hours could be demonstrated (Exp. 6).

Since heating alone for four hours at 120° produced no effect which could be demonstrated by the method of feeding adopted (*i.e.* with 15 % of fat in the dietary) and exposure to this temperature for even 12 hours produced relatively little, I have not troubled to test the effect of lower temperatures without aeration. Proof that fractions of the vitamine have been destroyed calls of course for experiments in which the total fat administered is reduced to the critical minimum.

With simultaneous aeration destruction of the vitamine proceeds with considerable rapidity at 80° (Exps. 2 and 7).

Very noteworthy are the results of full exposure to air at ordinary room temperatures. Butter exposed in thin layers at 15°–25° for periods of about a week was found to have lost its power of maintaining the growth or health of rats (Exp. 8). It is possible that in this experiment insufficient attention was paid to the accessory influence of light.

Aeration as is well known bleaches butter, but it now seems fully proved that the carotinoid pigments are not related to the fat-soluble A substance.

Though the hypothesis seems to me *prima facie* unlikely it may occur to some that combined heating and aeration might produce toxic products, or deleterious changes in the fat itself. In this connexion it is not without interest to point out that the general behaviour of the rats depending upon butter aerated at 120° for four hours or longer was very similar to that of the animals referred to earlier in this paper which depended on vegetable fats. In the latter growth continued on the average for 25 days and the average (from 60 rats) duration of life was 56 days. In the former the average period which elapsed before growth ceased was 23 days, and the average (from 42 rats) duration of life was 54 days. Aerated fat has frequently been added to the food of animals upon ordinary diet without deleterious effects.

With regard to the effect of the aeration upon the butter fats themselves, I may point out that Miss M. Stephenson has made determination of the iodine value of the fatty acids of butter before and after heating for four hours in a stream of air at 120°. The figures were identical (*e.g.* 39·0 before and 39·0 after).

Keratomalacia was for some reason more frequent in the animals upon aerated butter as just described than in those upon vegetable fats in the earlier investigation mentioned above. The latter formed part of the material used by Miss Stephenson and Miss Clark for their recently published [1920] study of the disorder. They found that only 28 % of the animals were affected. Taking all my animals upon butter aerated at 120° for four hours or longer one finds that just over 60 % showed symptoms of eye trouble. But although the complete absence of keratomalacia from the rats given heated, but unaerated, butter and its frequency upon the aerated fat is of interest as giving further proof that the loss of nutritive value from the latter is actually due to destruction of the fat-soluble A, I do not attach statistical importance to the above figure. The influence of stock, and perhaps of the nutrition preceding experiment, may affect the incidence of keratomalacia. In Exp. 4 (fat aerated four hours), and in Exp. 5 (aeration 12 hours), all the animals ultimately developed the condition. In Exp. 6, on the other hand, the animals upon butter aerated for four hours all remained free from it. The last were from a wholly different stock.

SUMMARY.

Experiments carried out upon a large number of rats have shown that the fat-soluble A substance in butter, while displaying marked resistance to heat alone at temperatures up to 120°, is readily destroyed by simultaneous aeration of the fat, presumably because it is a substance prone to oxidation by atmospheric oxygen.

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