LXX. RESEARCHES ON THE FAT-SOLUBLE ACCESSORY FACTOR (VITAMIN A). VI: EFFECT OF HEAT AND OXYGEN ON THE NUTRITIVE VALUE OF BUTTER.

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A number of somewhat conflicting statements regarding the influence of heat on the vitamin A are to be found in recent papers. Originally this substance was considered to be comparatively stable to high temperatures, for Osborne and Mendel [1915] had found that butter fat lost little or none of its growth-promoting properties when subjected to the action of live steam for two and one-half hours.

Later, however, experiments were recorded by Steenbock, Boutwell and Kent [1918], the results of which cast some doubt on the supposed stability of the vitamin. They found, for example, that butter aerated for 12 hours at a temperature of 100° completely lost its power to maintain growth. Naturally their first tendency was to ascribe the destruction to oxidation, but when they found that shaking melted butter fat with carbonated water for several hours caused inactivation of the vitamin just as readily as when the shaking was carried out in the presence of air, they were led to conclude that the heat alone was responsible.

A series of experiments on the effect of heat on the vitamin present in certain oils was carried out by Drummond [1919], who obtained results which appeared to support the conclusions of Steenbock and his collaborators. In these experiments reliance was placed upon observations of the iodine value in order to ascertain whether the destruction of the vitamin is a process of oxidation. The results indicated that oxidation was not the cause of destruction of this dietary factor at high temperatures. Early in 1920 a paper appeared by Osborne and Mendel [1920], in which they produced further evidence in favour of their view that the vitamin present in butter is stable to relatively high temperatures. They not only confirmed their earlier result on the treatment of butter fat with steam, but also found that a sample of butter fat heated to 96° for 15 hours retained its growth-promoting activity.

In view of their results it was obvious that the question required re-investigating, and accordingly we carried out a new series of experiments in order to seek the cause of the discrepancies.

The result of this work has been to establish that destruction of the growth factor A does take place with ease at high temperature, provided facilities for oxidation are present.

While these experiments were in progress we learnt from Professor Hopkins that he had also arrived at a similar conclusion [1920, 1, 2], and further confirmation is given by the experiments of Zilva [1920]. It is particularly interesting to note that Hopkins has observed that destruction of the vitamin at high temperatures may occur without an appreciable change in the iodine value of the accompanying oil.

Experimental.

Throughout these experiments we employed the technique described in a previous paper [Drummond and Coward, 1920]. The various samples of butter were tested by administering them to young rats whose growth had been completely inhibited by withholding the factor A from their diet. The daily ration of butter was always given separately to the animals before they received the day's ration of the basal diet. In order to be able to detect any destruction of the vitamin, the amount of the daily supplement was fixed at 0·2 g., a quantity which, in the case of the sample of butter used in these tests, was found to give slow but steady growth.

Effect of Exposure of Butter to High Temperatures.

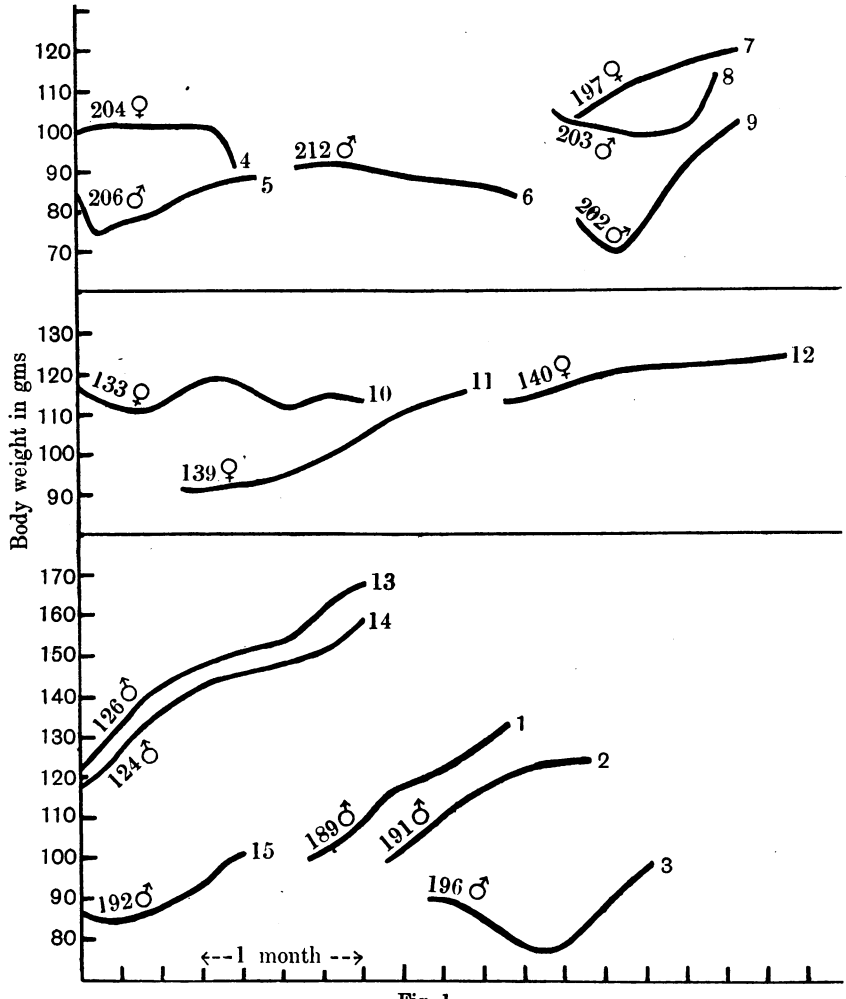
I. *Experiments at* 100°. A sample of the butter was subjected to the action of a current of live steam for six hours, an exposure longer by three and a half hours than that employed by Osborne and Mendel. Experiments on rats showed that this treatment had caused practically no loss of the growth-promoting factor A, an observation which is in agreement with that of the American workers. This treatment would of course effect the removal of the air in the vessel by the current of steam (Curves 1–3, Fig. 1).

II. *Experiments at* 96°. Quantities of butter were heated to 96° for 15 hours, as was done by Osborne and Mendel [1920]. One sample was placed in a large shallow dish in order to expose a considerable surface to the air, whilst another was enclosed during the heating in an air-free vessel. As may be seen from Fig. 1 destruction of the growth-promoting properties took place in the sample exposed to air (Curves 4–6) but not in that protected from oxidation (Curves 7–9).

Further experiments in which two samples of butter were heated to 96° for only three hours with and without air, respectively, gave similar results (Curves 10–12 and 13–15, Fig. 1).

III. *Experiments at* 50°. In order to re-investigate the effect of heating at comparatively low temperatures, another series of butter samples was heated for six hours at 50°. The results as a whole support the conclusion that oxidative processes are responsible for destruction of the vitamin A. As will

be seen from the Curves 16–18, Fig. 2, one animal (16) fed on the fat heated out of contact with air grew as if no deterioration in the nutritive value of the butter had occurred, but two others (17, 18) showed the opposite result. On the other hand, the animals fed on the exposed fat showed that this had been inactivated (Curves 19–21, Fig. 2).



N.B. *In all the curves presented in this paper the preliminary period when the rats were fed on the basal diet alone is omitted.*

A third sample of butter which was exposed to air in a vessel permitting a much smaller surface to come in contact with the atmosphere was only slightly inactivated (Curves 22–24, Fig. 2).

IV. *Experiments at* 37°. The most striking observation recorded in the earlier investigation [Drummond, 1919] was the instability of the vitamin A

at temperatures as low as 37°. It seemed, however, advisable to repeat the experiments employing the more satisfactory method of testing fractions which we now use. Four samples of the butter were heated at a temperature of 37° for a period of three weeks. Of these, two were exposed to air in thin layers in shallow glass dishes, whilst the other two were protected from air in closely stoppered vessels.

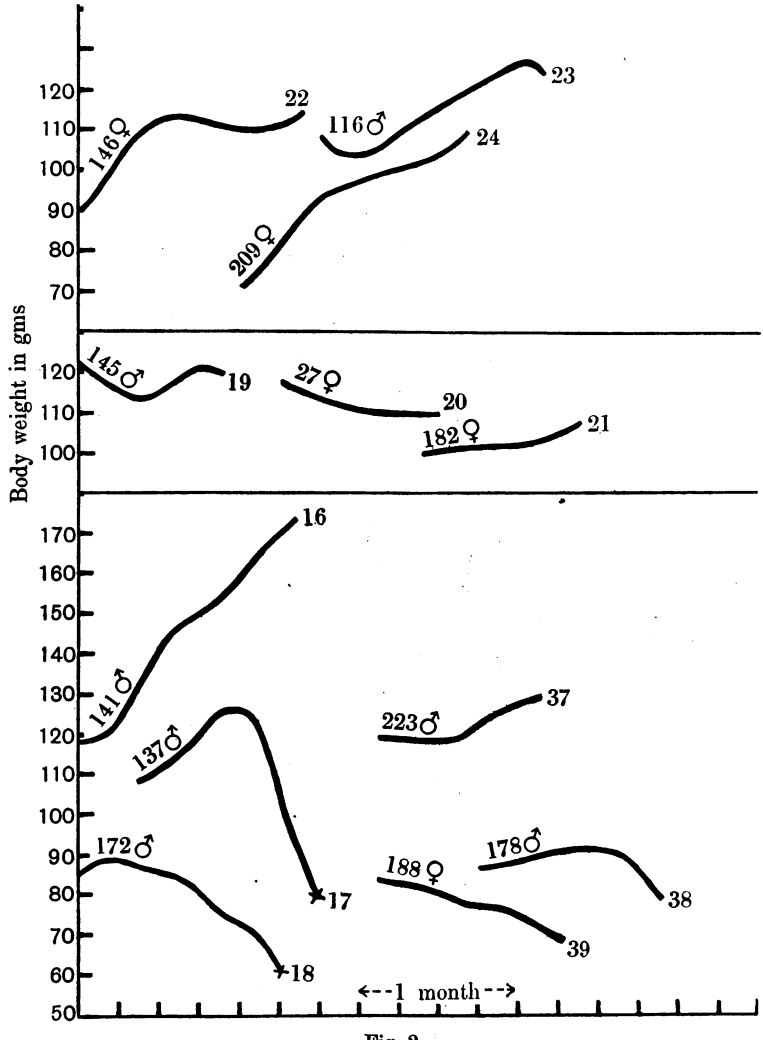


Fig. 2

One of each group was kept in a light corner of the laboratory, whilst the other two were placed in a darkened chamber. By this means it was hoped to ascertain the influence of light and air, since it has already been shown by Zilva [1919] that the vitamin A is destroyed on exposure to ultra-violet rays, a process which he suggested might be due to the action of the ozone

produced by the rays. Zilva has now obtained evidence that the cause of inactivation is oxidation by ozone [Zilva, 1920]. The samples exposed to air in this laboratory both became bleached, that kept in the light almost completely, and they were found at the end of three weeks to have lost their power of restoring growth in rats (Curves 25–27 and 32–34, Fig. 3). No appreciable impairment of the nutritive value of the samples protected from air was detected (Curves 28–30, and 31, 35, 36, Fig. 3).

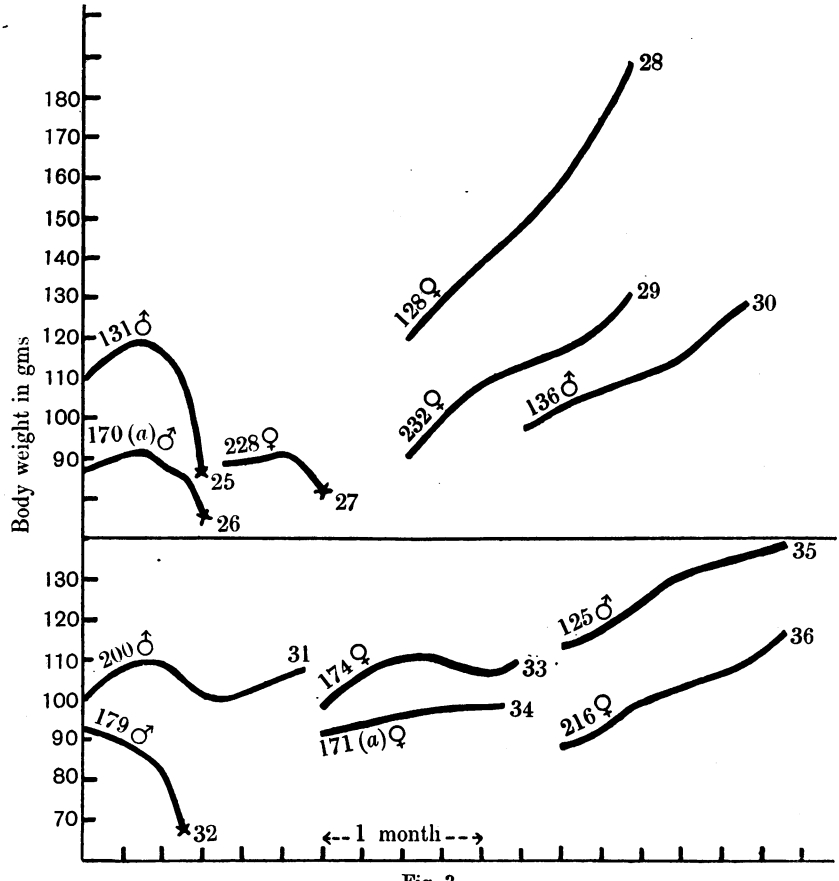


Fig. 3

In connection with these experiments we also tested a sample of butter fat, which Dr O. Rosenheim of King's College, London, very kindly placed at our disposal. This sample had during a previous investigation in 1917 been found to possess considerable growth-promoting power. It had been then dissolved in alcohol, and the solution allowed to stand for many months in the laboratory. When he sent it to us all the alcohol had evaporated, and there remained behind a perfectly white fat resembling a soft tallow. Tests on rats showed that the vitamin had been destroyed (Curves 37–39, Fig. 2).

SUMMARY.

1. Destruction of the vitamin present in butter fat occurs on heating in the presence of air. It is therefore probable that the loss is due to changes of an oxidative nature.

2. The destruction takes place rapidly at high temperatures, but provided the exposure to air is extensive, considerable loss of nutritive value may take place at temperatures as low as 37°.

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