CLXXVII. THE INFLUENCE OF SUNLIGHT ON MILK.

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LOJANDER [1925] noticed that exposure to sunlight decreased the time required to decolorise methylene blue in milk containing bacteria. The present author [1930] showed that sunlight caused a decoloration of methylene blue in milk independently of bacteria, and produced evidence in support of the theory that the reaction was accompanied by an oxidation of some portion of the milk fat.

Hammer and Cordes [1920] and Frazier [1928] had previously observed that whole milk exposed to sunlight developed a "cardboard" or tallowy flavour due to the oxidation of the milk fat. From the evidence available it seems probable that the two phenomena are based on the same chemical reaction so far as the fat is concerned, and that the presence of methylene blue in milk exposed to sunlight merely results in a visual indication of the intensity with which the fat oxidation is taking place. The author has observed that milk samples, exposed to sunlight with or without methylene blue, develop the tallowy flavour described by the American workers, and since neither the tallowy flavour nor the reduction of methylene blue occurs in milk from which the fat has been removed, it is almost certain that the two happenings are dependent upon one reaction—an oxidation-reduction phenomenon. The reaction appeared to be of sufficient interest to warrant a more detailed examination.

It has been shown by Clark *et al.* [1928] that the potential induced on an electrode of a noble metal immersed in a solution indicates the reducing or oxidising tendency more precisely and over a greater range than do the changes in colour undergone by a small amount of dye (such as methylene blue). The potentiometric method was applied, therefore, in an attempt to follow more fully the changes which take place in milk exposed to sunlight. Gold electrodes were immersed in tubes of milk. KCl-agar bridges connected the milk sample with a quinhydrone half cell prepared according to Veibel [1923]. The E.M.F. values given by such complete cells were measured at intervals by means of a potentiometer, and the E_h values calculated from the readings were plotted against time intervals. The milk used for the deter-

mination of the effect of sunlight was fresh and contained so few bacteria that it gave no appreciable change in E_h value within 7 hours when kept in darkness at 37°. In order to prevent the fat from rising too rapidly in the milk, the electrodes were moved gently up and down with a pumping action before taking each reading on the potentiometer, but they were never withdrawn wholly from the milk during the course of the experiment.

Whole milk.

When whole milk was exposed to sunlight, considerable changes in E_h were soon observed coincident with the decoloration of methylene blue in

Fig. 1. Whole milk in bright sunlight.

duplicate samples also exposed to light. Fig. 1 shows the curves obtained in several typical experiments.

The fact that a fall in E_h occurs in the absence of methylene blue indicates that the dye is not essential to the reaction and that there must be some reversible system in milk capable of acting as a hydrogen acceptor. Clark and Cohen [1928] have shown that this is also the case in the Schardinger reaction. The time required to bring about the change and also the extent of the change varied considerably with different milks, with the intensity of the sunlight, and also with the particular electrode used. It soon became evident that the reducing potentials induced in the milk were very poorly poised and that any attempt to make accurate measurements would be useless. Different electrodes in duplicate samples of milk under apparently identical conditions often showed differences of 50 millivolts in the potentiometer readings. The low

capacity of the reducing system formed during the fat oxidation was also evidenced by the fact that the presence of methylene blue (in dilution of 1 in 100,000) in milk in which the electrode was immersed resulted in a very definite tendency for the fall in potential to be checked in the region where methylene blue reduction occurs. In spite of this unavoidable inaccuracy, however, it was possible to gain some information from the general form of the curves which in a large number of experiments was fairly constant.

The form of curve obtained with whole milk exposed to sunlight is quite different from that obtained by plotting the changes of oxidation-reduction potential with time in milk in which bacteria are present in large numbers. In such cases the potential shows only a slight fall for some time and then drops very suddenly to a negative E_h value. The difference is easy to understand, for, when the action is due to bacteria, there is a rapid increase in the amount of enzyme (due to the multiplication of the organisms) during the course of the experiment, whereas in the action induced by sunlight there can be no increase in the amount of catalyst unless the intensity of the light increases markedly or the reaction is autocatalysed. The shape of the curve in the present experiments indicates that autocatalysis does not occur. In both types of reaction, however, the lower limit of E_h value reached appears to be about -0.2 v.

An apparent anomaly in the course of the reaction which was often evident was that in bright sunlight the methylene blue underwent decoloration while the E_h of the milk was still over $+0.2$ v., whereas the normal range for decoloration in aqueous solution is $+0.07$ to -0.01 v. at the p_H of milk. The reason for this has not been elucidated, but it seems very probable that, while the E_h of whole milk is changing rapidly, the potential on the electrode may lag in its indication of the conditions in the medium. This is especially likely to occur in the present case where the reaction responsible for the development of the reducing potential is taking place at the interface between two phases—the fat and the plasma. Some evidence that this lag in adjustment takes place was obtained by removing a tube of milk from bright sunlight to darkness midway in the reaction. When this was done, the potential on an electrode immersed in the milk continued to fall for some time, although it ceased to fall long before the potential in a duplicate tube exposed to sunlight the whole time. It seems more likely that in darkness the continued fall was due to an adjustment of the electrode to the actual conditions in the medium than that the reducing tendency continued to increase after the stimulus of sunlight was removed. Where the fall in potential was more gradual or less fat was present (as will be mentioned later), this decoloration of methylene blue at a potential apparently too high did not occur. In these cases also, removal of the tube from the influence of sunlight resulted in an immediate check in the fall in potential. It is suggested, therefore, that where the E_h falls more gradually or the medium is more nearly homogeneous, the potential on the electrode gives a more accurate indication of the conditions

in the medium from moment to moment. If this be the true explanation, it becomes evident that, under some conditions, a dye may give a truer indication of the oxidation-reduction potential of a solution than the potential on an electrode.

In early experiments the milk was maintained at 37° to 40° during exposure to sunlight. Owing to the inaccuracy of the potentiometric method, no attempt was made to determine the temperature coefficient of the reaction. It was found, however, that exposure of milk at room temperature resulted in a slowing of the reaction barely outside the limits of experimental error due to differences between electrodes. All later experiments were therefore performed at room temperature.

The reaction obtained with heated milk confirmed the results reported previously by the present author in that there was no essential difference from that obtained with raw milk.

Separated milk.

The results obtained by the exposure of separated milk to sunlight and measurement of the reducing potential were quite different from those obtained with whole milk and were not so regular. Many samples showed little change in E_h during the period of exposure to sunlight, this corresponding to the absence of change in methylene blue under such conditions. Other samples gave a decided fall similar to that obtained with whole milk, but the potential never fell much below zero E_h and then always showed a tendency to rise again to an E_h of between +0.2 and +0.3 v. This might be explained by assuming that the slight amount of residual fat (about 0.40 %) tended to undergo oxidation under the influence of the sunlight. The subsequent drift to a more positive potential can only be explained by assuming the presence in milk of some slowly reacting reducible system of large capacity, which in the oxidised form has an E_h of between +0.2 and +0.3 v. The presence of such a system in milk seems probable by reason of the fact that most milk samples, in the absence of influences such as sunlight and bacteria, attain equilibrium with a gold electrode at about this value of E_h . Fig. 2 gives several typical curves obtained with separated milk.

Although the curves obtained with some samples fell through the range where methylene blue reduction occurs, the dye was never reduced completely in separated milk. The reducing system had such a low capacity that the dye, when present, prevented by its poisoning action the extreme fall in E_h indicated in some of the curves. As has been mentioned before, a paling in colour sometimes took place with a subsequent deepening in colour as the potential rose again. The separated milk, moreover, never showed a continued fall in potential when the stimulus of sunlight was removed, and it is probable that the electrode was able to indicate the true potential of the medium more accurately from moment to moment. The occurrence or non-occurrence of a fall in potential in different batches of separated milk could not be correlated

with slight differences in fat content, and it is impossible at present to account satisfactorily for this variation in behaviour.

The effect of ultra-violet radiation.

Although in the foregoing experiments the sunlight passed through at least two layers of glass, the use of an ultra-violet spectroscope showed that some ultra-violet rays in sunlight were capable of reaching the milk.

In order to determine whether ultra-violet radiation was responsible for the stimulation of fat oxidation, experiments such as those already described were performed, replacing sunlight by radiation from a mercury vapour lamp. The tubes of milk were exposed at distances from the lamp varying from 1 ft.

Fig. 2. Separated milk in bright sunlight.

to 6 ft. Even at the shortest distance, no change in blue colour was observed in milk containing the dye, and only a very slow change in potential was observed until about 6 hours had elapsed from the beginning of irradiation. It appeared to take at least three times as long to produce any effect by means of ultra-violet radiation as by sunlight. Another difference was that the blue did not begin to fade until the E_h had fallen below zero, and it seems most probable that, owing to the slowness of the changes taking place, the electrode was able to maintain its adjustment with conditions in the milk, thus indicating more exactly the true potential from moment to moment than was the case in milk exposed to sunlight and undergoing rapid changes. Separated

milk gave results similar to those obtained in sunlight, but again the course of any reaction which occurred was much more prolonged. The agent inducing oxidation of fat in milk is therefore not ultra-violet radiation (or at least any of the ultra-violet rays most characteristic of the radiation from a mercury vapour lamp), but more probably some portion of visible light of which there is less in the radiation of the mercury vapour lamp than in sunlight. Trials with radiation from electric lamps were negative so far as any effect on oxidation of fat or reduction of methylene blue were concerned, and at present it is therefore impossible to define more accurately the particular type of radiation responsible for the reaction.

SUMMARY.

The oxidation of fat in whole milk under the influence of sunlight causes the development of a reducing potential, which can be detected either by its effect on methylene blue or by measurement electrometrically. The potential developed is poorly poised and of small capacity, hence experimental error in the measurement of potentials is great. Separated milk in many cases undergoes no change under the influence of sunlight, but some samples show a fall in potential which is not at present satisfactorily explained. When such a fall in potential occurs in separated milk there is a subsequent drift to more positive potentials. This suggests the presence in milk of a slowly acting reversible system which tends to maintain the E_h at a value between +0.2 and +0.3 v. It has not been found possible to reproduce the effect of sunlight by the use of either ultra-violet radiation or of radiation from electric lamps.

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