XIX. THE ACTION OF ULTRA-VIOLET RAYS ON THE ACCESSORY FOOD FACTORS.

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IN spite of the various attempts to isolate and identify the accessory food factors their chemical identity still remains unsolved. As far as the antineuritic and the antiscorbutic factors are concerned it is possible now by means of chemical fractionation to obtain residues which are very active. These preparations contain comparatively little impurity judging from the small quantities required to produce a therapeutic effect. Although the chemical aim has not been achieved the possibility of employing such active fractions has been found useful in furthering research on the accessory factors in the domain of nutrition.

As the chemical identification of these principles has not so far been successful by means of fractionating methods it becomes advisable to study their properties, because it contributes to the knowledge of the nature of these unknown substances. During the various investigations carried out on the accessory factors many of their properties have already been revealed. So far, however, the action of light on them has not been studied. This investigation is devoted to the study of ultra-violet rays on the accessory factors. These rays are well known to produce pronounced chemical changes in many compounds and also to influence the physiological activity of various biological principles, and it was therefore of interest to find out the effect on the accessory factors after an exposure to ultra-violet rays. The action of the light on the antiscorbutic, antineuritic and the fat-soluble *A* factor was investigated. As these factors cannot be obtained in pure form they could only be exposed to the ultra-violet rays as present in active substances. The antiscorbutic was used in the form of lemon juice from which the citric and other acids have been removed as described by Harden and Zilva [1918]. Autolysed yeast was used for the antineuritic and butter was employed as the source of the fat-soluble *A*.

EXPERIMENTAL.

The Antiscorbutic Factor.

In using the treated lemon juice for the antiscorbutic factor it was brought to a definite H-ion concentration in order to equalise the conditions. This was done colorimetrically by the addition of *N*/10 HCl to the juice and matched against a standard solution of glycine and *N*/10 HCl estimated by the gas

chain method to be P_H 2.34. The H-ion concentration was adjusted daily before the liquid was exposed to the ultra-violet light. The solution was introduced in a quartz tube of 1 inch diameter which was revolved by means of a water motor. As a source of ultra-violet rays the quartz mercury vapour lamp was used and placed about 6 inches from the revolving tube. The solution was exposed to the light for 8 hours. Three sets of animals were employed in this experiment. One set received the treated lemon juice without having its reaction adjusted, the other set received the treated lemon juice with its reaction adjusted as described above and the third set received the treated lemon juice brought to P_H 2.34 and exposed for 6 hours to the ultra-violet rays. Each set had four animals which received 0.5 cc., 1.5 cc., 2.5 cc. and 5 cc. of the respective solutions. Fig. 1 represents the weight curves of these animals.

Fig. 1

The animals in all the sets which received 0.5 cc. of the solution succumbed to scurvy within about 30–35 days. Of the animals receiving 1.5 cc. of the various solutions two died of scurvy within 42 days, and the third one receiving the treated lemon juice as a control was decreasing rapidly in weight when it was chloroformed on the 46th day. The post mortem revealed very marked scurvy. The guinea pigs which received 2.5 cc. of the solution all survived the duration of the experiment, namely 46 days. These animals were chloroformed and at the post mortem examination no signs of scurvy could be detected. The dose of 5 cc. also warded off scurvy entirely in all cases. These animals were growing when they were chloroformed after they had been under experiment for 46 days. They were found in good condition and no traces of scurvy could be detected.

One is bound to conclude from the above experiment that the exposure to the ultra-violet rays did not destroy or even impair the antiscorbutic activity of the treated lemon juice at the H-ion concentration of P_H 2.34.

An attempt was then made to ascertain whether the action of ultra-violet rays has any effect on the treated lemon juice in neutral condition. Lemon juice treated by the Harden and Zilva method is neutral or slightly acid to litmus; this reaction depends on how thoroughly the organic acids have been removed from the lemon juice. By taking the necessary precautions it is possible to prepare it always so that it reacts neutral to litmus. Such neutral preparations of lemon juice were used in this experiment. One group of guinea pigs received the exposed treated lemon juice; to the other group the unexposed treated lemon juice of the same batch was administered. As in the previous experiment doses of 0.5 cc., 1.5 cc., 2.5 cc. and 5 cc. were given. In this case too both animals receiving 0.5 cc. declined and died of scurvy within about a month, thus showing 0.5 cc. of the treated lemon juice to be a dose insufficient to delay or prevent the onset of scurvy in guinea-pigs. The dose of 1.5 cc. in both cases delayed but did not prevent the onset of scurvy. The animal receiving the solution exposed to the ultra-violet rays was chloroformed and found to be suffering from acute scurvy. The guinea pig which received the control solution died of scurvy after 33 days. 2.5 cc. and 5 cc. of both solutions was sufficient to prevent scurvy. The weight curves of the above animals (Fig. 2) show that no great differentiation can be established in the growth of the animals receiving similar doses of the respective solutions. It is therefore evident that the action of the ultra-violet rays produced no marked deterioration of the antiscorbutic potency of the neutral treated lemon juice.

The Antineuritic Factor.

In studying the influence of ultra-violet rays on the antineuritic accessory factor pigeons and rats were employed. A pigeon was kept on a diet of polished rice until it developed polyneuritis; a dose of the exposed autolysed yeast was then administered in order to see whether it had a curative effect. The

autolysed yeast was exposed to ultra-violet light in a similar manner to the lemon juice. The following protocol describes the experiment in detail.

Pigeon *H* showed decided symptoms of polyneuritis. Its head was bent well forward. The animal was unable to walk and could not stand firmly on its legs. 3 cc. of the exposed autolysed juice was administered per

Fig. 2

os at 2 p.m. and another 2 cc. at 4.30 p.m. At 6 p.m. the same afternoon an improvement was observed. The following day the animal was normal. It was kept on a rice diet and after 10 days it developed another attack of polyneuritis. 2 cc. only were administered this time. The animal nevertheless improved and kept up its condition for 6 days.

The above experiment shows that the exposure to the ultra-violet rays did not entirely destroy the antineuritic activity of the autolysed yeast. It was desirable to ascertain whether the antineuritic activity was at all impaired by the exposure to the light. Rats were found convenient animals for that purpose. These animals if kept on a diet free from the antineuritic do not grow and eventually die after about 6 weeks. If, however, the antineuritic is supplied after the deficiency has been enforced for several weeks normal growth is immediately resumed.

In this experiment three rats were employed. Their diet was made up as follows:

Basal mixture	10 g.
Antiscorbutic equivalent to lemon juice	5 cc.
Centrifuged butter-fat	1.8 g.
Olive oil	1.6 „

The basal mixture was made up of 75 % starch, 20 % caseinogen and 5 % salts.

Fig. 3. The first perpendicular dotted line marks the time at which the administration of the exposed juice was commenced and the second line that of the unexposed juice of the same batch.

As will be seen from Fig. 3, two of the rats (Nos. 228 and 229) showed no growth for $4\frac{1}{2}$ weeks; the third animal (No. 227) showed little growth, much below the normal. After $4\frac{1}{2}$ weeks No. 227 received in its daily ration 1 cc., No. 228 3 cc. and No. 229 5 cc. of autolysed yeast juice exposed to ultra-violet rays. All the three animals commenced growing. The improvement

was least marked in rat No. 227 which received only 1 cc. of juice, the dose being evidently insufficient. After 15 days the exposed autolysed juice was replaced by unexposed autolysed juice which belonged to the same batch, and as the curves show the growth in all the three cases proceeded at the same rate as before. This experiment shows that the exposure of autolysed yeast to ultra-violet rays for 6 hours neither destroys nor vitiates to any marked degree the antineuritic activity of the juice.

The Fat-soluble A Accessory.

The influence of the ultra-violet rays on the fat-soluble *A* factor was studied next. As butter was used for the source of this accessory factor the technique had to be somewhat modified. The butter was spread in a thin layer over a glass plate which was placed in a horizontal position about 6-8 inches from the lamp. The butter was turned over several times during the exposure. The part of the plate nearest the lamp became slightly warm from the heat of the lamp but it did not reach a higher temperature than 30°. It soon became apparent that the butter was greatly altered by exposure to the ultra-violet light. It became entirely bleached, acquired an odour like that of tallow, and was unpalatable and unfit for consumption. Several preliminary experiments showed that exposure to the ultra-violet rays destroyed the fat-soluble *A* in the butter and this was confirmed on repetition. The following experiments demonstrate it. The mode of procedure adopted was the same as that employed by Drummond [1919]. Three rats were placed on a diet free from the fat-soluble *A* factor, which was made up as follows:

Basal mixture	12 g.
Antiscorbutic equivalent to lemon juice	5 cc.
Autolysed yeast juice	5 „
Olive oil	0.75 cc.

Precautions were taken to destroy any traces of the fat-soluble *A* that might be present in the basal mixture by heating the starch and the caseinogen for 3 hours at 100° C., since it has been shown by Steenbock, Boutwell and Kent [1918] and Drummond [1919] that this factor is thermolabile. The animals were kept on this diet for 18 days after which time 2 g. of the exposed butter was added to the above daily ration. This diet was continued for 25 days, when the exposed butter in the diet was replaced for two rats by ordinary butter kept for 6 hours at 35°. The butter was kept at this temperature in order to demonstrate that the slight heating effect of the lamp, which, as already mentioned, never increased the temperature to more than 30° at the nearest point, was not responsible for the destruction of the factor. On examining Fig. 4 it is seen that during the first period when the rats were receiving the diet lacking the fat-soluble *A* factor two of the animals did not grow at all, the third animal did grow, but at a rate much below the normal. The addition of the exposed butter made no difference to the growth of either

of the animals. One of the rats left on that diet developed xerophthalmia and died after about 6 weeks. When the exposed butter was replaced by unexposed butter kept for 6 hours at 35° in the diet of the remaining two rats they at once commenced growing, thus showing that the fat-soluble *A* was supplied by the addition of the unexposed but not by the exposed butter. The fat-soluble *A* factor in the butter was therefore inactivated by exposure to the ultra-violet rays.

Fig. 4. The first perpendicular dotted line marks the time at which the administration of the exposed butter commenced and the second line that of the unexposed butter.

On exposing the butter to the ultra-violet rays by means of the technique described above two other factors are introduced which might be suspected to bring about the inactivation of the fat-soluble *A* factor, namely, (a) temperature and (b) ozone, which, as is well known, is produced by the action of ultra-violet light on the atmospheric oxygen. That the slight elevation of the temperature of the butter is not responsible for the inactivation is seen from the above experiment, as the addition to the diet of butter not exposed to the ultra-violet light but kept for 6 hours at 35° at once induces growth. The part played by the ozone in the inactivation of the factor, as well as the chemical changes produced by the ultra-violet light in the fat, are now being studied.

In this connection it is of interest to add that it has been previously pointed out by many investigators that the sterilisation of milk by means of ultra-violet rays imparts to the milk a peculiar taste. This is no doubt due to the alteration in the butter fat brought about by the exposure. In view of

the above observations one would also expect that the action of ultra-violet light on milk might greatly impair the activity of the fat-soluble factor in it and thus diminish its nutritive value, especially when used for the purpose of infant feeding.

SUMMARY.

1. The exposure of treated lemon juice to ultra-violet rays in neutral condition and at a H-ion concentration of P_H 2.34 for 8 hours does not influence its antiscorbutic activity.
2. The exposure of autolysed yeast juice for the same length of time does not impair its antineuritic potency.
3. Butter exposed for 8 hours to ultra-violet light undergoes a very noticeable change and the fat-soluble *A* factor in it becomes inactivated.

REFERENCES.

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